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THE UNITED STATES PHARMACOPEIAL CONVENTION  
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## SIX-MONTH IMPLEMENTATION GUIDELINE

The *United States Pharmacopeia–National Formulary* and its supplements become official six months after being released to the public. The *USP–NF*, which is released on November 1 of each year, becomes official on May 1 of the following year. This six-month implementation timing gives users more time to bring their methods and procedures into compliance with new and revised *USP–NF* requirements.

The table below describes the official dates of the *USP–NF* and its supplements. The 2016 *USP 39–NF 34*, and its supplements, *Interim Revision Announcements (IRAs)* and *Revision Bulletins* to that edition, will be official until May 1, 2017, at which time the *USP 40–NF 35* becomes official.

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<i>USP 40–NF 35</i>	November 1, 2016	May 1, 2017	May 1, 2018 (except as superseded by supplements, <i>IRAs</i> , and <i>Revision Bulletins</i> )
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# Guide to General Chapters

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# GENERAL NOTICES AND REQUIREMENTS

The *General Notices and Requirements* section (the *General Notices*) presents the basic assumptions, definitions, and default conditions for the interpretation and application of the *United States Pharmacopeia* (USP) and the *National Formulary* (NF).

Requirements stated in these *General Notices* apply to all articles recognized in the USP and NF (the "compendia") and to all general chapters unless specifically stated otherwise.

## 1. TITLE AND REVISION

The full title of this publication (consisting of four volumes and including its *Supplements*), is *The Pharmacopeia of the United States of America*, Fortieth Revision and the *National Formulary*, Thirty-Fifth Edition. These titles may be abbreviated to USP 40, to NF 35, and to USP 40–NF 35. The *United States Pharmacopeia*, Fortieth Revision, and the *National Formulary*, Thirty-Fifth Edition, supersede all earlier revisions. Where the terms "USP," "NF," or "USP–NF" are used without further qualification during the period in which these compendia are official, they refer only to USP 40, NF 35, and any *Supplement(s)* thereto. The same titles, with no further distinction, apply equally to print or electronic presentation of these contents. Although USP and NF are published under one cover and share these *General Notices*, they are separate compendia.

This revision is official beginning May 1, 2017 unless otherwise indicated in specific text.

*Supplements* to USP and NF are published periodically.

Accelerated Revisions, published periodically on the *Official Text* section of USP's website (<http://www.usp.org/usp-nf/official-text>), are designed to make revisions official more quickly than through the routine process for publishing standards in the USP–NF. *Interim Revision Announcements* are Accelerated Revisions to USP and NF that contain official revisions and their effective dates.

*Revision Bulletins* are Accelerated Revisions to official text or postponements that require expedited publication. They generally are official immediately unless otherwise specified in the *Revision Bulletin*.

*Errata* are Accelerated Revisions representing corrections to items erroneously published. Announcements of the availability of new USP Reference Standards and announcements of tests or procedures that are held in abeyance pending availability of required USP Reference Standards are also available on the "Official Text" tab of USP's website.

## 2. OFFICIAL STATUS AND LEGAL RECOGNITION

### 2.10. Official Text

Official text of the USP and NF is published in the USP–NF Online ([www.uspnf.com](http://www.uspnf.com)) in the edition identified as "CURRENTLY OFFICIAL" and in Accelerated Revisions that supersede the USP–NF Online as described below.

Routine revisions are published in the USP–NF Online and become official on the date indicated, usually six months after publication. Accelerated Revisions supersede the USP–NF Online and become official on the date indicated. Links to Accelerated Revisions on the USP website can be found in any superseded monograph or general chapter in the USP–NF Online.

Print and USB flash drive versions of the USP and NF also are available. Routine revisions are provided with the same timing as the USP–NF Online. Official text published in *Supplements* supersedes that in the previously published print or

USB flash drive versions of USP–NF. These versions also are superseded by Accelerated Revisions as described above.

In the event of any disparity between the print or USB flash drive versions and the USP–NF Online, the USP–NF Online will be deemed to apply.

### 2.20. Official Articles

An *official article* is an article that is recognized in USP or NF. An article is deemed to be recognized and included in a compendium when a monograph for the article is published in the compendium and an official date is generally or specifically assigned to the monograph.

The title specified in a monograph is the *official title* for such article. Other names considered to be synonyms of the official titles may not be used as substitutes for official titles.

*Official articles* include both *official substances* and *official products*. An *official substance* is a drug substance, excipient, dietary ingredient, other ingredient, or component of a finished device for which the monograph title includes no indication of the nature of the finished form.

An *official product* is a drug product, dietary supplement, compounded preparation, or finished device for which a monograph is provided.

### 2.30. Legal Recognition

The USP and NF are recognized in the laws and regulations of many countries throughout the world. Regulatory authorities may enforce the standards presented in the USP and NF, but because recognition of the USP and NF may vary by country, users should understand applicable laws and regulations. In the United States under the Federal Food, Drug, and Cosmetic Act (FDCA), both USP and NF are recognized as official compendia. A drug with a name recognized in USP–NF must comply with compendial identity standards or be deemed adulterated, misbranded, or both. See, e.g., FDCA § 501(b) and 502(e)(3)(b); also FDA regulations, 21 CFR § 299.5(a&b). To avoid being deemed adulterated, such drugs must also comply with compendial standards for strength, quality, and purity, unless labeled to show all respects in which the drug differs. See, e.g., FDCA § 501(b) and 21 CFR § 299.5(c). In addition, to avoid being deemed misbranded, drugs recognized in USP–NF must also be packaged and labeled in compliance with compendial standards. See FDCA § 502(g).

A dietary supplement represented as conforming to specifications in USP will be deemed a misbranded food if it fails to so conform. See FDCA § 403(s)(2)(D).

Enforcement of USP standards is the responsibility of FDA and other government authorities in the U.S. and elsewhere. USP has no role in enforcement.

### Change to read:

## 3. CONFORMANCE TO STANDARDS

### 3.10. Applicability of Standards

Standards for an article recognized in the compendia (USP–NF) are expressed in the article's monograph, applicable general chapters, and *General Notices*. The identity, strength, quality, and purity of an article are determined by the official tests, procedures, and acceptance criteria, and other requirements incorporated in the monograph, in applicable general chapters, or in the *General Notices*. "Applicable general chapters" means general chapters numbered



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below 1000 or above 2000 that are made applicable to an article through reference in *General Notices*, a monograph, or another applicable general chapter numbered below 1000. Where the requirements of a monograph differ from the requirements specified in these *General Notices* or an applicable general chapter, the monograph requirements apply and supersede the requirements of the *General Notices* or applicable general chapters, whether or not the monograph explicitly states the difference.

General chapters numbered 1000 to 1999 are for informational purposes only. They contain no mandatory tests, assays, or other requirements applicable to any official article, regardless of citation in a general chapter numbered below 1000, a monograph, or these *General Notices*. General chapters numbered above 2000 apply only to articles that are intended for use as dietary ingredients and dietary supplements. General chapter citations in *NF* monographs refer to *USP* general chapters.

Early adoption of revised standards in advance of the official date is allowed by *USP* unless specified otherwise at the time of publication. Where revised standards for an existing article have been published as final approved "official text" (as approved in section 2.10 *Official Text*) but have not yet reached the official date (six months after publication, unless otherwise specified; see "official date", section 2.20 *Official Articles*), compliance with the revised standard shall not preclude a finding or indication of conformance with compendial standards, unless *USP* specifies otherwise by prohibiting early adoption in a particular standard.

The standards in the relevant monograph, general chapter(s), and *General Notices* apply at all times in the life of the article from production to expiration. It is also noted that the manufacturer's specifications, and manufacturing practices (e.g., Quality by Design, Process Analytical Technology, and Real Time Release Testing initiatives), generally are followed to ensure that the article will comply with compendial standards until its expiration date, when stored as directed. Every compendial article in commerce shall be so constituted that when examined in accordance with these assays and test procedures, it meets all applicable pharmacopeial requirements (*General Notices*, monographs, and general chapters). Thus, any official article is expected to meet the compendial standards if tested, and any official article actually tested as directed in the relevant monograph must meet such standards to demonstrate compliance.

Some tests, such as those for Dissolution and Uniformity of Dosage Units, require multiple dosage units in conjunction with a decision scheme. These tests, albeit using a number of dosage units, are in fact one determination. These procedures should not be confused with statistical sampling plans. The similarity to statistical procedures may seem to suggest an intent to make inference to some larger group of units, but in all cases, statements about whether the compendial standard is met apply only to the units tested. Repeats, replicates, statistical rejection of outliers, or extrapolations of results to larger populations, as well as the necessity and appropriate frequency of batch testing, are neither specified nor proscribed by the compendia; such decisions are based on the objectives of the testing. Frequency of testing and sampling are left to the preferences or direction of those performing compliance testing, and other users of *USP-NF*, including manufacturers, buyers, or regulatory authorities.

Official products are prepared according to recognized principles of good manufacturing practice and from ingredi-

ents that meet *USP* or *NF* standards, where standards for such ingredients exist (for dietary supplements, see section 3.10.20).

Official substances are prepared according to recognized principles of good manufacturing practice and from ingredients complying with specifications designed to ensure that the resultant substances meet the requirements of the compendial monographs.

### 3.10.10. Applicability of Standards to Drug Products, Drug Substances, and Excipients

The applicable *USP* or *NF* standard applies to any article marketed in the United States that (1) is recognized in the compendium and (2) is intended or labeled for use as a drug or as an ingredient in a drug. Such articles (drug products, drug substances, and excipients) include both human drugs (whether dispensed by prescription, "over the counter," or otherwise), as well as animal drugs. The applicable standard applies to such articles whether or not the added designation "*USP*" or "*NF*" is used. The standards apply equally to articles bearing the official titles or names derived by transposition of the definitive words of official titles or transposition in the order of the names of two or more active ingredients in official titles, or where there is use of synonyms with the intent or effect of suggesting a significant degree of identity with the official title or name.

### 3.10.20. Applicability of Standards to Medical Devices, Dietary Supplements, and Their Components and Ingredients

An article recognized in *USP* or *NF* shall comply with the compendial standards if the article is a medical device, component intended for a medical device, dietary supplement, dietary ingredient, or other ingredient that is intended for incorporation into a dietary supplement, and is labeled as conforming to the *USP* or *NF*.

Generally, dietary supplements are prepared from ingredients that meet *USP*, *NF*, or *Food Chemicals Codex* standards. Where such standards do not exist, substances may be used in dietary supplements if they have been shown to be of acceptable food grade quality using other suitable procedures.

### 3.10.30. Applicability of Standards to the Practice of Compounding (New)

*USP* compounding practice standards, *Pharmaceutical Compounding—Nonsterile Preparations* (795) and *Pharmaceutical Compounding—Sterile Preparations* (797), as appropriate, apply to compounding practice or activity regardless of whether a monograph exists for the compounded preparation or these chapters are referenced in such a monograph. In the United States, (795) and (797) are not applicable to drugs compounded by entities registered with FDA as outsourcing facilities as defined by FDCA § 503B, because such facilities are required to comply with FDA's current good manufacturing practice requirements. Compounded preparations, including drug products compounded by outsourcing facilities, may also be subject to applicable monographs; see section 2.20 *Official Articles* and section 4.10 *Monographs*. ▲*USP40*

### 3.20. Indicating Conformance

A drug product, drug substance, or excipient may use the designation "*USP*" or "*NF*" in conjunction with its official title or elsewhere on the label only when (1) a monograph



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is provided in the specified compendium and (2) the article complies with the identity prescribed in the specified compendium.

When a drug product, drug substance, compounded preparation, or excipient differs from the relevant *USP* or *NF* standard of strength, quality, or purity, as determined by the application of the tests, procedures, and acceptance criteria set forth in the relevant compendium, its difference shall be plainly stated on its label.

When a drug product, drug substance, compounded preparation, or excipient fails to comply with the identity prescribed in *USP* or *NF* or contains an added substance that interferes with the prescribed tests and procedures, the article shall be designated by a name that is clearly distinguishing and differentiating from any name recognized in *USP* or *NF*.

A medical device, dietary supplement, or ingredient or component of a medical device or dietary supplement may use the designation "USP" or "NF" in conjunction with its official title or elsewhere on the label only when (1) a monograph is provided in the specified compendium and (2) the article complies with the monograph standards and other applicable standards in that compendium.

The designation "USP" or "NF" on the label may not and does not constitute an endorsement by USP and does not represent assurance by USP that the article is known to comply with the relevant standards. USP may seek legal redress if an article purports to be or is represented as an official article in one of USP's compendia and such claim is determined by USP not to be made in good faith.

The designation "USP-NF" may be used on the label of an article provided that the label also bears a statement such as "Meets NF standards as published by USP," indicating the particular compendium to which the article purports to apply.

When the letters "USP," "NF," or "USP-NF" are used on the label of an article to indicate compliance with compendial standards, the letters shall appear in conjunction with the official title of the article. The letters are not to be enclosed in any symbol such as a circle, square, etc., and shall appear in capital letters.

If a dietary supplement does not comply with all applicable compendial requirements but contains one or more dietary ingredients or other ingredients that are recognized in *USP* or *NF*, the individual ingredient(s) may be designated as complying with *USP* or *NF* standards or being of *USP* or *NF* quality provided that the designation is limited to the individual ingredient(s) and does not suggest that the dietary supplement complies with *USP* standards.

#### 4. MONOGRAPHS AND GENERAL CHAPTERS

##### 4.10. Monographs

Monographs set forth the article's name, definition, specification, and other requirements related to packaging, storage, and labeling. The specification consists of tests, procedures, and acceptance criteria that help ensure the identity, strength, quality, and purity of the article. For general requirements relating to specific monograph sections, see section 5 *Monograph Components*.

Because monographs may not provide standards for all relevant characteristics, some official substances may conform to the *USP* or *NF* standard but differ with regard to nonstandardized properties that are relevant to their use in specific preparations. To assure substitutability in such instances, users may wish to ascertain functional equivalence or determine such characteristics before use.

##### 4.10.10. Applicability of Test Procedures

A single monograph may include more than one test, procedure, and/or acceptance criterion for the same attribute. Unless otherwise specified in the monograph, all tests are requirements. In some cases, monograph instructions allow the selection of tests that reflect attributes of different manufacturers' articles, such as different polymorphic forms, impurities, hydrates, and dissolution. Monograph instructions

indicate the tests, procedures, and/or acceptance criteria to be used and the required labeling.

The order in which the tests are listed in the monograph is based on the order in which they are approved by the relevant Expert Committee for inclusion in the monograph. Test 1 is not necessarily the test for the innovator or for the reference product. Depending on monograph instructions, a labeling statement is not typically required if Test 1 is used.

##### 4.10.20. Acceptance Criteria

The acceptance criteria allow for analytical error, for unavoidable variations in manufacturing and compounding, and for deterioration to an extent considered acceptable under practical conditions. The existence of compendial acceptance criteria does not constitute a basis for a claim that an official substance that more nearly approaches 100 percent purity "exceeds" compendial quality. Similarly, the fact that an article has been prepared to tighter criteria than those specified in the monograph does not constitute a basis for a claim that the article "exceeds" the compendial requirements.

An official product shall be formulated with the intent to provide 100 percent of the quantity of each ingredient declared on the label. Where the minimum amount of a substance present in a dietary supplement is required by law to be higher than the lower acceptance criterion allowed for in the monograph, the upper acceptance criterion contained in the monograph may be increased by a corresponding amount.

The acceptance criteria specified in individual monographs and in the general chapters for compounded preparations are based on such attributes of quality as might be expected to characterize an article compounded from suitable bulk drug substances and ingredients, using the procedures provided or recognized principles of good compounding practice, as described in these compendia.

##### 4.20. General Chapters

Each general chapter is assigned a number that appears in angle brackets adjacent to the chapter name (e.g., *Chromatography* (621)). General chapters may contain the following:

- Descriptions of tests and procedures for application through individual monographs,
- Descriptions and specifications of conditions and practices for pharmaceutical compounding,
- General information for the interpretation of the compendial requirements,
- Descriptions of general pharmaceutical storage, dispensing, and packaging practices, or
- General guidance to manufacturers of official substances or official products.

When a general chapter is referenced in a monograph, acceptance criteria may be presented after a colon.

Some chapters may serve as introductory overviews of a test or of analytical techniques. They may reference other general chapters that contain techniques, details of the procedures, and, at times, acceptance criteria.

#### Change to read:

#### 5. MONOGRAPH COMPONENTS

##### 5.10. Molecular Formula

The use of the molecular formula for the active ingredient(s) named in defining the required strength of a compendial article is intended to designate the chemical entity or entities, as given in the complete chemical name of the article, having absolute (100 percent) purity.

##### 5.20. Added Substances

Added substances are presumed to be unsuitable for inclusion in an official article and therefore prohibited, if their presence impairs the bioavailability, therapeutic efficacy, or safety of the official article; or they interfere with the assays and tests prescribed for determining compliance with the



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compendial standards (see section 3.20 *Indicating Conformance*).

The air in a container of an official article may, where appropriate, be evacuated or be replaced by carbon dioxide, helium, argon, or nitrogen, or by a mixture of these gases. The use of such gas need not be declared in the labeling.

#### 5.20.10. Added Substances in Official Substances

Official substances may contain only the specific added substances that are permitted by the individual monograph. Such added substances shall not exceed the quantity required for providing their intended effect. Where such addition is permitted, the label shall indicate the name(s) and amount(s) of any added substance(s).

#### 5.20.20. Added Substances (Excipients and Ingredients) in Official Products

Suitable substances and excipients such as antimicrobial agents, pharmaceutical bases, carriers, coatings, flavors, preservatives, stabilizers, and vehicles may be added to an official product to enhance its stability, usefulness, or elegance, or to facilitate its preparation, unless otherwise specified in the individual monograph.

Added substances and excipients employed solely to impart color may be incorporated into official products other than those intended for parenteral or ophthalmic use, in accordance with the regulations pertaining to the use of colors issued by the U.S. Food and Drug Administration (FDA), provided such added substances or excipients are otherwise appropriate in all respects. (See also *Injectables and Implanted Drugs Products (1)*, *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Vehicles and added substances*, *Added substances*.) USP40

The proportions of the substances constituting the base in ointment and suppository products and preparations may be varied to maintain a suitable consistency under different climatic conditions, provided that the concentrations of active ingredients are not varied and provided that the bioavailability, therapeutic efficacy, and safety of the preparation are not impaired.

#### 5.20.20.1. In Compounded Preparations

Compounded preparations for which a complete composition is given shall contain only the ingredients named in the formulas unless specifically exempted herein or in the individual monograph. Deviation from the specified processes or methods of compounding, although not from the ingredients or proportions thereof, may occur provided that the finished preparation conforms to the relevant standards and to preparations produced by following the specified process.

Where a monograph for a compounded preparation calls for an ingredient in an amount expressed on the dried basis, the ingredient need not be dried before use if due allowance is made for the water or other volatile substances present in the quantity taken.

Specially denatured alcohol formulas are available for use in accordance with federal statutes and regulations of the Internal Revenue Service. A suitable formula of specially denatured alcohol may be substituted for Alcohol in the manufacture of official preparations intended for internal or topical use, provided that the denaturant is volatile and does not remain in the finished product. A preparation that is intended for topical application to the skin may contain specially denatured alcohol, provided that the denaturant is either a usual ingredient in the preparation or a permissible added substance; in either case the denaturant shall be identified on the label of the topical preparation. Where a process is given in the individual monograph, any preparation compounded using denatured alcohol shall be identical to that prepared by the monograph process.

#### 5.20.20.2. In Dietary Supplements

Additional ingredients may be added to dietary supplement products provided that the additional ingredients: (1) comply with applicable regulatory requirements; and (2) do

not interfere with the assays and tests prescribed for determining compliance with compendial standards.

#### 5.30. Description and Solubility

Only where a quantitative solubility test is given in a monograph and is designated as such is it a test for purity.

A monograph may include information regarding the article's description. Information about an article's "description and solubility" also is provided in the reference table *Description and Relative Solubility of USP and NF Articles*. The reference table merely denotes the properties of articles that comply with monograph standards. The reference table is intended primarily for those who use, prepare, and dispense drugs and/or related articles. Although the information provided in monographs and the information in the reference table may indirectly assist in the preliminary evaluation of an article, it is not intended to serve as a standard or test for purity.

The approximate solubility of a compendial substance is indicated by one of the following descriptive terms:

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1,000
Very slightly soluble	From 1,000 to 10,000
Practically insoluble, or Insoluble	Greater than or equal to 10,000

#### 5.40. Identity

A compendial test titled *Identity* or *Identification* is provided as an aid in verifying the identity of articles as they are purported to be, e.g., those taken from labeled containers, and to establish whether it is the article named in *USP-NF*. The *Identity* or *Identification* test for a particular article may consist of one or more procedures. When a compendial test for *Identity* or *Identification* is undertaken, all requirements of all specified procedures in the test must be met to satisfy the requirements of the test. Failure of an article to meet all the requirements of a prescribed *Identity* or *Identification* test (i.e., failure to meet the requirements of all of the specified procedures that are components of that test) indicates that the article is mislabeled and/or adulterated.

#### 5.50. Assay

Assay tests for compounded preparations are not intended for evaluating a compounded preparation before dispensing, but instead are intended to serve as the official test in the event of a question or dispute regarding the preparation's conformance to official standards.

#### 5.50.10. Units of Potency (Biological)

For substances that cannot be completely characterized by chemical or physical means or that need confirmation of functionality or tertiary structure, it may be necessary to express quantities of biological activity in units of biological potency, each defined by an authoritative, designated reference standard. In cases where international reference materials have been discontinued, international units of potency may be defined in terms of molecular mass, such as in the cases of vitamins A, D, and E.

Where available, World Health Organization (WHO) international biological standards define the International Units (IU). *USP* monographs refer to the units assigned by *USP Reference Standards* either directly as International Units (IU) or as "USP Units." For some biological products, units of potency are value assigned against a corresponding U.S. Standard established by FDA, whether or not International Units or USP Units have been defined (see *Biologics* (1041)). Note that product-related labeling, e.g., on containers, need not use the full phrase "USP [product name] Units" that



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appears in many USP monograph labeling sections. The term "USP Units" can be used on product labeling consistent with USP compendial requirements, provided it is clear from the context that the volume is stated in terms of USP [product name] Units. In such circumstances it should be clear that "USP Units" and "USP [product name] Units" share the same meaning.

#### 5.60. Impurities and Foreign Substances

Tests for the presence of impurities and foreign substances are provided to limit such substances to amounts that are unobjectionable under conditions in which the article is customarily employed (see also *Impurities in Drug Substances and Drug Products* (1086)).

Nonmonograph tests and acceptance criteria suitable for detecting and controlling impurities that may result from a change in the processing methods or that may be introduced from external sources should be employed in addition to the tests provided in the individual monograph, where the presence of the impurity is inconsistent with applicable good manufacturing practices or good pharmaceutical practices.

#### 5.60.10. Other Impurities in USP and NF Articles

If a USP or NF monograph includes an assay or organic impurity test based on chromatography, other than a test for residual solvents, and that monograph procedure does not detect an impurity present in the substance, the amount and identity of the impurity, where both are known, shall be stated in the labeling (certificate of analysis) of the official substance, under the heading *Other Impurity(ies)*.

The presence of any unlabeled other impurity in an official substance is a variance from the standard if the content is 0.1% or greater. The sum of all *Other Impurities* combined with the monograph-detected impurities may not exceed 2.0% (see *Ordinary Impurities* (466)), unless otherwise stated in the monograph.

The following categories of drug substances are excluded from *Other Impurities* requirements:

- Fermentation products and semi-synthetics derived therefrom,
- Radiopharmaceuticals,
- Biologics,
- Biotechnology-derived products,
- Peptides,
- Herbals, and
- Crude products of animal or plant origin.

Any substance known to be toxic shall not be listed under *Other Impurities*.

#### 5.60.20. Residual Solvents in USP and NF Articles

All USP and NF articles are subject to relevant control of residual solvents, even when no test is specified in the individual monograph. If solvents are used during production, they must be of suitable quality. In addition, the toxicity and residual level of each solvent shall be taken into consideration, and the solvents limited according to the principles defined and the requirements specified in *Residual Solvents* (467), using the general methods presented therein or other suitable methods.

#### 5.60.30. Elemental Impurities in USP Drug Products and Dietary Supplements

Effective January 1, 2018, elemental impurities will be controlled in official drug products according to the principles defined and requirements specified in *Elemental Impurities—Limits* (232). Effective January 1, 2018, elemental contaminants are controlled in official dietary supplements according to the principles defined and requirements specified in *Elemental Contaminants in Dietary Supplements* (2232). Also effective January 1, 2018, *Heavy Metals* (231) will be omitted and all references to it in general chapters and monographs will be deleted. Early adoption of the requirements in (232) and (2232) are permitted by USP, and if (232) or (2232), as applicable, is fully implemented with respect to a particular drug product or dietary supplement in advance of the January 1, 2018 date, that product and its ingredients will no longer need to comply with applicable

(231) requirements to be considered by USP to be in conformance with USP–NF requirements.

#### 5.70. Performance Tests

Where content uniformity determinations have been made using the same analytical methodology specified in the Assay, with appropriate allowances made for differences in sample preparation, the average of all of the individual content uniformity determinations may be used as the Assay value.

#### 5.80. USP Reference Standards

USP Reference Standards are authentic specimens that have been approved as suitable for use as comparison standards in USP or NF tests and assays. (See *USP Reference Standards* (11).) Where USP or NF tests or assays call for the use of a USP Reference Standard, only those results obtained using the specified USP Reference Standard are conclusive. Where a procedure calls for the use of a compendial article rather than for a USP Reference Standard as a material standard of reference, a substance meeting all of the compendial monograph requirements for that article shall be used. If any new USP or NF standard requires the use of a new USP Reference Standard that is not yet available, that portion of the standard containing the requirement shall not be official until the specified USP reference material is available.

Unless a Reference Standard label bears a specific potency or content, assume the Reference Standard is 100.0% pure in the official application. Unless otherwise directed in the procedure in the individual monograph or in a general chapter, USP Reference Standards are to be used in accordance with the instructions on the label of the Reference Standard.

#### Change to read:

### 6. TESTING PRACTICES AND PROCEDURES

#### 6.10. Safe Laboratory Practices

In performing compendial procedures, safe laboratory practices shall be followed, including precautionary measures, protective equipment, and work practices consistent with the chemicals and procedures used. Before undertaking any procedure described in the compendia, the analyst should be aware of the hazards associated with the chemicals and the techniques and means of protecting against them. These compendia are not designed to describe such hazards or protective measures.

#### 6.20. Automated Procedures

Automated and manual procedures employing the same basic chemistry are considered equivalent.

#### 6.30. Alternative and Harmonized Methods and Procedures

Alternative methods and/or procedures may be used if they provide advantages in terms of accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, or in other special circumstances. Such alternative procedures and methods shall be validated as described in *Validation of Compendial Procedures* (1225) and must be shown to give equivalent or better results. Only those results obtained by the methods and procedures given in the *compendia* are conclusive.

Alternative procedures should be submitted to USP for evaluation as a potential replacement or addition to the standard (see section 4.10 *Monographs*).

Certain general chapters contain a statement that the text in question is harmonized with the corresponding text of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia* and that these texts are interchangeable. Therefore, if a substance or preparation is found to comply with a requirement using an interchangeable method or procedure from one of these pharmacopoeias, it should comply with the requirements of the USP–NF. When a difference appears, or in the event of dispute, only the result obtained by the method and/or procedure given in the USP–NF is conclusive.



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**6.40. Dried, Anhydrous, Ignited, or Solvent-Free Basis**

All calculations in the compendia assume an "as-is" basis unless otherwise specified.

Test procedures may be performed on the undried or unignited substance and the results calculated on the dried, anhydrous, or ignited basis, provided a test for *Loss on Drying*, or *Water Determination*, or *Loss on Ignition*, respectively, is given in the monograph. Where the presence of moisture or other volatile material may interfere with the procedure, previous drying of the substance is specified in the individual monograph and is obligatory.

The term "solvent-free" signifies that the calculation shall be corrected for the presence of known solvents as determined using the methods described in (467) unless a test for limit of organic solvents is provided in the monograph.

The term "previously dried" without qualification signifies that the substance shall be dried as directed under *Loss on Drying* (731) or *Water Determination* (921) (gravimetric determination).

Where drying in vacuum over a desiccant is directed, a vacuum desiccator, a vacuum drying pistol, or other suitable vacuum drying apparatus shall be used.

**6.40.10. Ignite to Constant Weight**

"Ignite to constant weight" means that ignition shall be continued at  $800 \pm 25^\circ$ , unless otherwise indicated, until two consecutive weighings, the second of which is taken after an additional period appropriate to the nature and quantity of the residue, do not differ by more than 0.50 mg per g of substance taken.

**6.40.20. Dried to Constant Weight**

"Dried to constant weight" means that drying shall be continued until two consecutive weighings, the second of which is taken after an additional drying period appropriate to the nature and quantity of the residue, do not differ by more than 0.50 mg per g of substance taken.

**6.50. Preparation of Solutions****6.50.10. Filtration**

Where a procedure gives direction to "filter" without further qualification, the liquid shall be passed through suitable filter paper or equivalent device until the filtrate is clear. Due to the possibility of filter effects, the initial volumes of a filtrate may be discarded.

**6.50.20. Solutions**

Unless otherwise specified, all solutions shall be prepared with Purified Water. Solutions for quantitative measures shall be prepared using accurately weighed or accurately measured analytes (see section 8.20 *About*).

An expression such as "(1 in 10)" means that 1 part by volume of a liquid shall be diluted with, or 1 part by weight of a solid shall be dissolved in, a sufficient quantity of the diluent or solvent to make the volume of the finished solution 10 parts by volume. An expression such as "(20:5:2)" means that the respective numbers of parts, by volume, of the designated liquids shall be mixed, unless otherwise indicated.

**6.50.20.1. Adjustments to Solutions**

When a specified concentration is called for in a procedure, a solution of other normality or molarity may be used, provided that allowance is made for the difference in concentration and that the change does not increase the error of measurement.

Proportionately larger or smaller quantities than the specified weights and volumes of assay or test substances and Reference Standards may be taken, provided the measurement is made with at least equivalent accuracy.

Unless otherwise indicated, analyte concentrations shall be prepared to within ten percent (10%) of the indicated value. In the case in which a procedure is adapted to the working range of an instrument, solution concentrations may differ from the indicated value by more than ten percent (10%), with appropriate changes in associated calculations. Any changes shall fall within the validated range of the instrument.

When adjustment of pH is indicated with either an acid or base and the concentration is not indicated, appropriate concentrations of that acid or base may be used.

**6.50.20.2. Test Solutions**

Information on Test Solutions (TS) is provided in the *Test Solutions* portion of the *Reagents, Indicators, and Solutions* section of the *USP-NF*. Use of an alternative Test Solution or a change in the Test Solution used may require validation.

**6.50.20.3. Indicator Solutions**

Where a procedure specifies the use of an indicator TS, approximately 0.2 mL, or 3 drops, of the solution shall be added unless otherwise directed.

**6.60. Units Necessary to Complete a Test**

Unless otherwise specified, a sufficient number of units to ensure a suitable analytical result shall be taken.

**6.60.10. Tablets**

Where the procedure of a Tablet monograph directs to weigh and finely powder not fewer than a given number of Tablets, a counted number of Tablets shall be weighed and reduced to a powder. The portion of the powdered Tablets taken shall be representative of the whole Tablets and shall, in turn, be weighed accurately.

**6.60.20. Capsules**

Where the procedure of a Capsule monograph gives direction to remove, as completely as possible, the contents of not fewer than a given number of the Capsules, a counted number of Capsules shall be carefully opened and the contents quantitatively removed, combined, mixed, and weighed accurately. The portion of mixed Capsules contents taken shall be representative of the contents of the Capsules and shall, in turn, be weighed accurately.

**6.70. Reagents**

The proper conduct of the compendial procedures and the reliability of the results depend, in part, upon the quality of the reagents used in the performance of the procedures. Unless otherwise specified, reagents conforming to the specifications set forth in the current edition of *Reagent Chemicals* published by the American Chemical Society (ACS) shall be used. Where such ACS reagent specifications are not available or where the required purity differs, compendial specifications for reagents of acceptable quality are provided (see the *Reagents, Indicators, and Solutions* section of the *USP-NF*). Reagents not covered by any of these specifications should be of a grade suitable to the proper performance of the method of assay or test involved.

Listing of these reagents, including the indicators and solutions employed as reagents, in no way implies that they have therapeutic utility; furthermore, any reference to *USP* or *NF* in their labeling shall include also the term "reagent" or "reagent grade." *USP* may supply reagents if they otherwise may not be generally commercially available.

**6.80. Equipment**

Unless otherwise specified, a specification for a definite size or type of container or apparatus in a procedure is given solely as a recommendation. Other dimensions or types may be used if they are suitable for the intended use.

**6.80.10. Apparatus for Measurement**

Where volumetric flasks or other exact measuring, weighing, or sorting devices are specified, this or other equipment of at least equivalent accuracy shall be employed.

**6.80.10.1. Pipet/Pipette**

Where a pipet/pipette is specified, a suitable buret may be substituted. Where a "to contain" pipet/pipette is specified, a suitable volumetric flask may be substituted.

**6.80.10.2. Light Protection**

Where low-actinic or light-resistant containers are specified, either containers specially treated to protect contents from light or clear containers that have been rendered opaque by application of a suitable coating or wrapping may be used.



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**6.80.20. Instrumental Apparatus**

An instrument may be substituted for the specified instrument if the substitute uses the same fundamental principles of operation and is of equivalent or greater sensitivity and accuracy. These characteristics shall be qualified as appropriate. Where a particular brand or source of a material, instrument, or piece of equipment, or the name and address of a manufacturer or distributor, is mentioned (ordinarily in a footnote), this identification is furnished solely for informational purposes as a matter of convenience, without implication of approval, endorsement, or certification.

**6.80.20.1. Chromatographic Tubes and Columns**

The term "diameter" refers to internal diameter (ID).

**6.80.20.2. Tubing**

The term "diameter" refers to outside diameter (OD).

**6.80.20.3. Steam Bath**

Where use of a steam bath is directed, use actively flowing steam or another regulated heat source controlled at an equivalent temperature.

**6.80.20.4. Water Bath**

A water bath requires vigorously boiling water unless otherwise specified.

**6.80.30. Temperature Reading Devices**

Temperature reading devices suitable for pharmacopeial tests conform to specifications that are traceable to a National Institute of Standards and Technology (NIST) standard or equivalent. Temperature reading devices may be of the liquid-in-glass type or an analog or digital temperature indicator type, such as a resistance temperature device, thermistor, or thermocouple. Standardization of thermometers is performed on an established testing frequency with a temperature standard traceable to NIST. For example, refer to the current issue of American Society of Testing and Materials (ASTM) standards E1 for liquid-in-glass thermometers.

**7. TEST RESULTS****7.10. Interpretation of Requirements**

Analytical results observed in the laboratory (or calculated from experimental measurements) are compared with stated acceptance criteria to determine whether the article conforms to compendial requirements.

The reportable value, which often is a summary value for several individual determinations, is compared with the acceptance criteria. The reportable value is the end result of a completed measurement procedure, as documented.

Where acceptance criteria are expressed numerically herein through specification of an upper and/or lower limit, permitted values include the specified values themselves, but no values outside the limit(s). Acceptance criteria are considered significant to the last digit shown.

**7.10.5. Nominal Concentrations in Equations**

Where a "nominal concentration" is specified, calculate the concentration based on the label claim. In assay procedures, water correction is typically stated in the Definition

and on the label of the USP Reference Standard. For other procedures, correction for assayed content, potency, or both is made prior to using the concentration in the equation provided in the monograph.

**7.10.10. Equivalence Statements in Titrimetric Procedures**

The directions for titrimetric procedures conclude with a statement of the weight of the analyte that is equivalent to each mL of the standardized titrant. In such an equivalence statement, the number of significant figures in the concentration of the titrant should be understood to correspond to the number of significant figures in the weight of the analyte. Corrections to calculations based on the blank determination are to be made for all titrimetric assays where appropriate (see *Titrimetry* (541)).

**7.20. Rounding Rules**

The observed or calculated values shall be rounded off to the number of decimal places that is in agreement with the limit expression. Numbers should not be rounded until the final calculations for the reportable value have been completed. Intermediate calculations (e.g., slope for linearity) may be rounded for reporting purposes, but the original (not rounded) value should be used for any additional required calculations. Acceptance criteria are fixed numbers and are not rounded.

When rounding is required, consider only one digit in the decimal place to the right of the last place in the limit expression. If this digit is smaller than 5, it is eliminated and the preceding digit is unchanged. If this digit is equal to or greater than 5, it is eliminated and the preceding digit is increased by 1.

**Change to read:** *When rounding is required, consider only one digit in the decimal place to the right of the last place in the limit expression. If this digit is smaller than 5, it is eliminated and the preceding digit is unchanged. If this digit is equal to or greater than 5, it is eliminated and the preceding digit is increased by 1.*

**8. TERMS AND DEFINITIONS****8.10. Abbreviations**

- RS refers to a USP Reference Standard.
- CS refers to a Colorimetric Solution.
- TS refers to a Test Solution.
- VS refers to a Volumetric Solution that is standardized in accordance with directions given in the individual monograph or in the *Reagents, Indicators, and Solutions* section of USP-NF.

**8.20. About**

"About" indicates a quantity within 10%.

If the measurement is stated to be "accurately measured" or "accurately weighed," follow the statements in *Volumetric Apparatus* (31) and *Balances* (41), respectively.

**8.30. Alcohol Content**

Percentages of alcohol, such as those under the heading *Alcohol Content*, refer to percentage by volume of  $C_2H_5OH$  at 15.56°. Where a formula, test, or assay calls for alcohol, ethyl alcohol, or ethanol, the USP monograph article Alcohol

**Illustration of Rounding Numerical Values  
for Comparison with Requirements**

Compendial Requirement	Unrounded Value	Rounded Result	Conforms
Assay limit $\geq 98.0\%$	97.96%	98.0%	Yes
	97.92%	97.9%	No
	97.95%	98.0%	Yes
Assay limit $\leq 101.5\%$	101.55%	101.6%	No
	101.46%	101.5%	Yes
	101.45%	101.5%	Yes
Limit test $\leq 0.02\%$	0.025%	0.03%	No
	0.015%	0.02%	Yes
	0.027%	0.03%	No
Limit test $\leq 3$ ppm	3.5 ppm	4 ppm	No
	3.4 ppm	3 ppm	Yes
	2.5 ppm	3 ppm	Yes



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shall be used. Where reference is made to " $C_2H_5OH$ ," absolute (100 percent) ethanol is intended. Where a procedure calls for dehydrated alcohol, alcohol absolute, or anhydrous alcohol, the USP monograph article Dehydrated Alcohol shall be used.

**8.40. Atomic Weights**

Atomic weights used in computing molecular weights and the factors in the assays and elsewhere are those established by the IUPAC Commission on Isotopic Abundances and Atomic Weights. ▲USP40

**8.50. Blank Determinations**

Where it is directed that "any necessary correction" be made by a blank determination, the determination shall be conducted using the same quantities of the same reagents treated in the same manner as the solution or mixture containing the portion of the substance under assay or test, but with the substance itself omitted.

**8.60. Concomitantly**

"Concomitantly" denotes that the determinations or measurements are to be performed in immediate succession.

**8.70. Desiccator**

The instruction "in a desiccator" indicates use of a tightly closed container of suitable size and design that maintains an atmosphere of low moisture content by means of a suitable desiccant such as anhydrous calcium chloride, magnesium perchlorate, phosphorus pentoxide, or silica gel. See also section 8.220 *Vacuum Desiccator*.

**8.80. Logarithms**

Logarithms are to the base 10.

**8.90. Microbial Strain**

A microbial strain cited and identified by its American Type Culture Collection (ATCC) catalog number shall be used directly or, if subcultured, shall be used not more than five passages removed from the original strain.

**8.100. Negligible**

"Negligible" indicates a quantity not exceeding 0.50 mg.

**8.110. NLT/NMT**

"NLT" means "not less than." "NMT" means "not more than."

**8.120. Odor**

"Odorless," "practically odorless," "a faint characteristic odor," and variations thereof indicate evaluation of a suitable quantity of freshly opened material after exposure to the air for 15 minutes. An odor designation is descriptive only and should not be regarded as a standard of purity for a particular lot of an article.

**8.130. Percent**

"Percent" used without qualification means:

- For mixtures of solids and semisolids, percent weight in weight;
- For solutions or suspensions of solids in liquids, percent weight in volume;
- For solutions of liquids in liquids, percent volume in volume;
- For solutions of gases in liquids, percent weight in volume.

For example, a 1 percent solution is prepared by dissolving 1 g of a solid or semisolid, or 1 mL of a liquid, in sufficient solvent to make 100 mL of the solution.

**8.140. Percentage Concentrations**

Percentage concentrations are expressed as follows:

- *Percent Weight in Weight (w/w)* is defined as the number of g of a solute in 100 g of solution.
- *Percent Weight in Volume (w/v)* is defined as the number of g of a solute in 100 mL of solution.
- *Percent Volume in Volume (v/v)* is defined as the number of mL of a solute in 100 mL of solution.

**8.150. Pressure**

Pressure is determined by use of a suitable manometer or barometer calibrated in terms of the pressure exerted by a column of mercury of the stated height.

**8.160. Reaction Time**

Reaction time is 5 minutes unless otherwise specified.

**8.170. Specific Gravity**

Specific gravity is the weight of a substance in air at 25° divided by the weight of an equal volume of water at the same temperature.

**8.180. Temperatures**

Temperatures are expressed in centigrade (Celsius) degrees, and all measurements are made at 25° unless otherwise indicated. Where moderate heat is specified, any temperature not higher than 45° (113° F) is indicated.

**8.190. Time**

Unless otherwise specified, rounding rules, as described in section 7.20 *Rounding Rules*, apply to any time specified.

**8.200. Transfer**

"Transfer" indicates a quantitative manipulation.

**8.210. Vacuum**

"Vacuum" denotes exposure to a pressure of less than 20 mm of mercury (2.67 kPa), unless otherwise indicated.

**8.220. Vacuum Desiccator**

"Vacuum desiccator" indicates a desiccator that maintains a low-moisture atmosphere at a reduced pressure of not more than 20 mm of mercury (2.67 kPa) or at the pressure designated in the individual monograph.

**8.230. Water****8.230.10. Water as an Ingredient in an Official Product**

As an ingredient in an official product, water meets the requirements of the appropriate water monograph in USP or NF.

**8.230.20. Water in the Manufacture of Official Substances**

When used in the manufacture of official substances, water shall meet the requirements for drinking water as set forth in the U.S. Environmental Protection Agency National Primary Drinking Water Regulations or in the drinking water regulations of the European Union or of Japan, or in the World Health Organization's Guidelines for Drinking Water Quality. Additional specifications may be required in monographs.

**8.230.30. Water in a Compendial Procedure**

When water is called for in a compendial procedure, the USP article Purified Water shall be used unless otherwise specified. Definitions for other types of water are provided in *Reagents, Indicators, and Solutions* and in *Water for Pharmaceutical Purposes* (1231).

**8.240. Weights and Measures**

In general, weights and measures are expressed in the International System of Units (SI) as established and revised by the *Conférence générale des poids et mesures*. For compendial purposes, the term "weight" is considered to be synonymous with "mass."

Molality is designated by the symbol *m* preceded by a number that represents the number of moles of the designated solute contained in 1 kilogram of the designated solvent.

Molarity is designated by the symbol *M* preceded by a number that represents the number of moles of the designated solute contained in an amount of the designated solvent that is sufficient to prepare 1 liter of solution.

Normality is designated by the symbol *N* preceded by a number that represents the number of equivalents of the designated solute contained in an amount of the designated solvent that is sufficient to prepare 1 liter of solution.

The symbol for degrees (°) without a qualifying unit of measure represents degrees Celsius.

Chart of Symbols and Prefixes commonly employed for SI metric units and other units:

	Units	Symbol	Notes
Length			
	meter	m	



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	Units	Symbol	Notes
	centimeter	cm	
	millimeter	mm	
	micrometer	μm	Previously referred to as a micron
	nanometer	nm	Previously the symbol mμ (for millimicron) was used
	Ångström	Å	Equal to 0.1 nm
Mass			
	kilogram	kg	
	gram	g	
	milligram	mg	
			The symbol μg is used in the USP and NF to represent micrograms, but micrograms may be represented as "mcg" for labeling and prescribing purposes. The term "gamma," symbolized by γ, frequently is used to represent micrograms in biochemical literature.
	microgram	μg	
	nanogram	ng	
	pico gram	pg	
			Also referred to as the unified atomic mass unit and is equal to 1/12 times the mass of the free carbon 12 atom.
	dalton	Da	
	kilodalton	kDa	
Time			
	second	s	
	minute	min	
	hour	h	
Volume			
	liter	L	1 L is equal to 1000 cm <sup>3</sup> (cubic centimeters)
	deciliter	dL	
	milliliter	mL	1 mL is equal to 1 cm <sup>3</sup> , sometimes referred to as cc
	microliter	μL	
Temperature			
	Celsius	°C	
Amount of Substance			
			Historically referred to as gram-molecular weight or gram-atomic weight
	mole	mol	
	millimole	mmol	
	micromole	μmol	
	femtomole	fmol	

	Units	Symbol	Notes
			Also referred to as gram-equivalent weight. It is used in the calculation of substance concentration in units of normality. This unit is no longer preferred for use in analytical chemistry or metrology.
	equivalent	Eq	
	milli equivalent	mEq	
			Osmotic pressure of a solution, related to substance concentration.
	osmole	Osmol	
	milliosmole	mOsmol	
Pressure			
	pascal	Pa	
	kilopascal	kPa	
	pounds per square inch	psi	
	millimeter of mercury	mmHg	Equal to 133.322 Pa
Electrical units			
	ampere	A	
	volt	V	
	millivolt	mV	
	hertz	Hz	Unit of frequency
	kilohertz	kHz	
	megahertz	MHz	
	electron volt	eV	
	kilo-electron volt	keV	
	mega-electron volt	MeV	
Radiation			
	becquerel	Bq	SI unit of activity for radionuclides
	kilobecquerel	kBq	
	megabecquerel	MBq	
	gigabecquerel	GBq	
	curie	Ci	Non-SI unit of activity for radionuclides
	millicurie	mCi	
	microcurie	μCi	
	nanocurie	nCi	
Other			
	acceleration due to gravity	g	Used to express rate of centrifugation
	revolutions per minute	rpm	Used to express rate of centrifugation

## Selected SI Prefixes

Name	Symbol	Factor
giga	G	10 <sup>9</sup>
mega	M	10 <sup>6</sup>



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Selected SI Prefixes (Continued)

Name	Symbol	Factor
kilo	k	$10^3$
deci	d	$10^{-1}$
centi	c	$10^{-2}$
milli	m	$10^{-3}$
micro	$\mu$	$10^{-6}$
nano	n	$10^{-9}$
pico	p	$10^{-12}$
femto	f	$10^{-15}$

**9. PRESCRIBING AND DISPENSING****9.10. Use of Metric Units**

Prescriptions for compendial articles shall be written to state the quantity and/or strength desired in metric units unless otherwise indicated in the individual monograph (see also section 5.50.10 *Units of Potency [Biological]* above). If an amount is prescribed by any other system of measurement, only an amount that is the metric equivalent of the pre-

scribed amount shall be dispensed. Abbreviations for the terms "Units" or "International Units" shall not be used for labeling or prescribing purposes. Apothecary unit designations on labels and labeling shall not be used.

**9.20. Changes in Volume**

In the dispensing of prescription medications, slight changes in volume owing to variations in room temperatures may be disregarded.

**10. PRESERVATION, PACKAGING, STORAGE, AND LABELING****10.10. Packaging and Storage**

All articles in *USP* or *NF* are subject to the packaging and storage requirements specified in *Packaging and Storage Requirements* (659), unless different requirements are provided in an individual monograph.

**10.20. Labeling**

All articles in *USP* or *NF* are subject to the labeling requirements specified in *Labeling* (7), unless different requirements are provided in an individual monograph.



# Official Monographs for USP 40

## Abacavir Oral Solution

### DEFINITION

Abacavir Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of abacavir ( $C_{14}H_{18}N_6O$ ).

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Solution A:** Trifluoroacetic acid and water (0.05:99.95)

**Solution B:** Methanol and water (17:3)

**Diluent:** 1 mL of phosphoric acid diluted with water to 1000 mL

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	70	30
35	10	90
40	10	90
41	0	100
50	0	100
51	95	5
55	95	5

**System suitability solution:** 0.2 mg/mL of USP Abacavir System Suitability Mixture RS in *Diluent*

**Standard solution:** 0.46 mg/mL of USP Abacavir Sulfate RS in *Diluent*

**Sample solution:** Equivalent to 0.4 mg/mL of abacavir in *Diluent*, from Oral Solution. [NOTE—Sonicate, if necessary.]

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 15-cm; 5-μm packing L1

**Column temperature:** 30°

**Flow rate:** 0.8 mL/min

**Injection size:** 10 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between abacavir and *trans*-abacavir, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{14}H_{18}N_6O$  in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak area of abacavir from the *Sample solution*  
 $r_S$  = peak area of abacavir from the *Standard solution*

$C_S$  = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of abacavir in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of abacavir multiplied by 2, 572.66

$M_{r2}$  = molecular weight of abacavir sulfate, 670.74

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

- DELIVERABLE VOLUME (698):** Meets the requirements

### IMPURITIES

#### Organic Impurities

##### PROCEDURE

**Solution A, Solution B, Diluent, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Sensitivity solution:** 0.2 μg/mL of USP Abacavir Sulfate RS in *Diluent*, from the *Standard solution*. [NOTE—The concentration of this solution is 0.05% of the nominal concentration of the *Sample solution*.]

#### Analysis

**Samples:** *Diluent, Standard solution, Sample solution, and Sensitivity solution*. [NOTE—In the *Sample solution* disregard any peaks corresponding to peaks identified in the *Diluent* and any peak with a peak area less than the abacavir peak area in the *Sensitivity solution*.] Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak area of abacavir from the *Sample solution*  
 $r_S$  = peak area of abacavir from the *Standard solution*

$C_S$  = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of abacavir in the *Sample solution* (mg/mL)

$F$  = relative response factor for each impurity from *Impurity Table 1*

$M_{r1}$  = molecular weight of abacavir multiplied by 2, 572.66

$M_{r2}$  = molecular weight of abacavir sulfate, 670.74

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 2.0%



Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cyclopropyldiaminopurine abacavir <sup>a</sup>	0.57	1.4	0.3
Descyclopropyl abacavir <sup>b</sup>	0.68	1.0	0.8
Abacavir	1.00	—	—
<i>trans</i> -Abacavir <sup>c</sup>	1.04	1.0	—
Any individual unspecified impurity	—	1.0	0.2

<sup>a</sup> N<sup>6</sup>-Cyclopropyl-9H-purine-2,6-diamine.<sup>b</sup> [(1*S*,4*R*)-4-(2,6-Diamino-9H-purin-9-yl)cyclopent-2-en-1-yl]methanol.<sup>c</sup> [(1*R*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-cyclopent-2-en-1-yl]methanol. It is a process impurity and monitored in the drug substance.**SPECIFIC TESTS**

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed 100 cfu/mL, and the total combined molds and yeast count does not exceed 10 cfu/mL. It also meets the requirement for absence of *Escherichia coli*.

- PH (791):** 3.8–4.5

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**  
USP Abacavir Sulfate RS  
USP Abacavir System Suitability Mixture RS  
A mixture containing abacavir sulfate and *trans*-abacavir

**Abacavir Tablets****DEFINITION**

Abacavir Tablets contain Abacavir Sulfate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of abacavir (C<sub>14</sub>H<sub>18</sub>N<sub>6</sub>O).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****PROCEDURE**

**Diluent:** 1.0 mL of phosphoric acid in 1 L of water

**Solution A:** Trifluoroacetic acid and water (0.05:99.95)

**Solution B:** Methanol and water (85:15)

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	70	30
35	10	90
40	10	90
41	95	5
50	95	5

**System suitability solution:** 0.2 mg/mL of USP Abacavir System Suitability Mixture RS in *Diluent*

**Standard solution:** 0.21 mg/mL of abacavir sulfate in *Diluent* (equivalent to 0.18 mg/mL of abacavir), from USP Abacavir Sulfate RS

**Sample stock solution:** Transfer the equivalent to 1500 mg of abacavir, from a portion of Tablets, into a 250-mL volumetric flask. Add 150 mL of *Diluent*. Shake mechanically for 45 min. Dilute with *Diluent* to volume. Pass a portion through a suitable filter of 0.45-μm or finer pore size. Discard the first 3 mL of the filtrate.

**Sample solution:** 0.18 mg/mL of abacavir in *Diluent* using the filtrate obtained in the *Sample stock solution*

**Chromatographic system**

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 15-cm; packing L1

**Flow rate:** 0.8 mL/min

**Injection volume:** 10 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between abacavir and *trans*-abacavir, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of abacavir (C<sub>14</sub>H<sub>18</sub>N<sub>6</sub>O) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of abacavir from the *Sample solution*

$r_S$  = peak response of abacavir from the *Standard solution*

$C_S$  = concentration of abacavir sulfate in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of abacavir in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of abacavir multiplied by 2, 572.66

$M_{r2}$  = molecular weight of abacavir sulfate, 670.74

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****DISSOLUTION (711)**

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 15 min

**Standard solution:** 0.39 mg/mL of USP Abacavir Sulfate RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** 254 nm

**Blank:** *Medium*

Calculate the percentage of the labeled amount of abacavir (C<sub>14</sub>H<sub>18</sub>N<sub>6</sub>O) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$M_{r1}$  = molecular weight of abacavir multiplied by 2, 572.66

$M_{r2}$  = molecular weight of abacavir sulfate, 670.74

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of abacavir (C<sub>14</sub>H<sub>18</sub>N<sub>6</sub>O) is dissolved.



- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

**Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

#### Analysis

[NOTE—Record the chromatograms for 2.5 times the retention time of abacavir.]

**Samples:** *Standard solution* and *Sample solution*

- Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of abacavir from the *Standard solution*

$C_S$  = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of abacavir in the *Sample solution* (mg/mL)

$F$  = relative response factor for each impurity (see *Table 2*)

$M_{r1}$  = molecular weight of abacavir multiplied by 2, 572.66

$M_{r2}$  = molecular weight of abacavir sulfate, 670.74

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cyclopropylidaminopurine abacavir <sup>a</sup>	0.57	1.4	0.2
Desacyclopropyl abacavir <sup>b</sup>	0.68	1.0	0.2
Abacavir	1.0	—	—
<i>trans</i> -Abacavir <sup>c,d</sup>	1.04	—	—
O-Pyrimidine derivative abacavir <sup>d,e</sup>	1.24	—	—
Any other individual impurity	—	1.0	0.2
Total impurities	—	—	1.0

<sup>a</sup> N<sup>6</sup>-Cyclopropyl-9H-purine-2,6-diamine.

<sup>b</sup> [(1*S*,4*R*)-4-(2,6-Diamino-9H-purin-9-yl)-cyclopent-2-enyl]methanol.

<sup>c</sup> [(1*R*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-cyclopent-2-enyl]methanol.

<sup>d</sup> Process impurity monitored in the drug substance and not included in the total impurities.

<sup>e</sup> N<sup>6</sup>-Cyclopropyl-9-[(1*R*,4*S*)-4-[(2,5-diamino-6-chloropyrimidin-4-yl)oxymethyl]cyclopent-2-enyl]-9H-purine-2,6-diamine.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Abacavir Sulfate RS  
USP Abacavir System Suitability Mixture RS—A mixture of abacavir sulfate and *trans*-abacavir.

## Abacavir and Lamivudine Tablets

### DEFINITION

Abacavir and Lamivudine Tablets contain an amount of abacavir sulfate and lamivudine equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of abacavir (C<sub>14</sub>H<sub>18</sub>N<sub>6</sub>O) and NLT 90.0% and NMT 110.0% of the labeled amount of lamivudine (C<sub>8</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>S), respectively.

### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Diluent:** 0.1 N hydrochloric acid

**Solution A:** Water and trifluoroacetic acid (2000:1)

**Solution B:** Acetonitrile, methanol, and trifluoroacetic acid (1000:1000:1)

**Mobile phase:** See *Table 1*. [NOTE—Return to original conditions and re-equilibrate the system for about 7 min.]

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
12	70	30
12.1	40	60
13.1	40	60
13.2	100	0

**System suitability solution:** Dissolve the contents of one vial of USP Lamivudine Resolution Mixture C RS in 2.5 mL of *Diluent*. [NOTE—One vial of USP Lamivudine Resolution Mixture C RS contains 0.8 mg of USP Lamivudine Resolution Mixture C RS.]

**Standard solution:** 0.35 mg/mL of USP Abacavir Sulfate RS and 0.15 mg/mL of USP Lamivudine RS in *Diluent*. Sonicate to dissolve prior to final dilution.

**Sample stock solution:** Nominally 3 mg/mL of abacavir and 1.5 mg/mL of lamivudine in *Diluent* prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask. Add *Diluent* to about 50% of the final volume and shake for NMT 30 min to disperse the Tablets. Dilute with *Diluent* to volume. Pass through a suitable filter.

**Sample solution:** Nominally 0.3 mg/mL of abacavir and 0.15 mg/mL of lamivudine in *Diluent* from *Sample stock solution*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm × 15-cm; 3.5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for lamivudine-S-oxide and lamivudine-R-oxide, in relation to the lamivudine peak, are 0.31 and 0.36, respectively; the relative retention times for lamivudine diastereomer and lamivudine are 0.88 and 1.0, respectively; *System suitability solution*.]

#### Suitability requirements

**Resolution:** NLT 1.0 between lamivudine-S-oxide and lamivudine-R-oxide; NLT 1.0 between lamivudine



diastereomer and lamivudine, *System suitability solution*

**Relative standard deviation:** NMT 1.5% each for abacavir and lamivudine, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of abacavir ( $C_{14}H_{18}N_6O$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of abacavir from the *Sample solution*

$r_S$  = peak response of abacavir from the *Standard solution*

$C_S$  = concentration of USP Abacavir Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of abacavir in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of abacavir multiplied by 2, 572.66

$M_{r2}$  = molecular weight of abacavir sulfate, 670.74

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of abacavir

Calculate the percentage of the labeled amount of lamivudine ( $C_8H_{11}N_3O_3S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of lamivudine from the *Sample solution*

$r_S$  = peak response of lamivudine from the *Standard solution*

$C_S$  = concentration of USP Lamivudine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of lamivudine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of lamivudine

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Standard solution 1:** 0.79 mg/mL of USP Abacavir Sulfate RS in *Medium*. Sonicate to dissolve prior to final dilution.

**Standard solution 2:** 0.33 mg/mL of USP Lamivudine RS in *Medium*. Sonicate to dissolve prior to final dilution.

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

##### Instrumental conditions

**Mode:** UV

**Wavelength:** 240–320 nm

**Cell length:** 0.2 mm

**Blank:** *Medium*

**Analysis:** The calculations of the percentages dissolved are performed using multi-component analysis software.

**Tolerances:** NLT 80% (Q) of the labeled amount of abacavir and lamivudine is dissolved.

##### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

Diluent, *Solution A*, *Solution B*, *Mobile phase*, *System suitability solution*, *Standard solution*, *Sample stock solution*, *Sample solution*, *Chromatographic system*, and *System suitability*: Proceed as directed in the Assay.

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each individual abacavir related impurity and each unspecified impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each abacavir related impurity or unspecified impurity

$r_T$  = sum of the peak responses of abacavir, all abacavir related impurities, and all unspecified impurities

Calculate the percentage of each lamivudine related impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each lamivudine related impurity

$r_T$  = sum of the peak responses of lamivudine and all lamivudine related impurities

**Acceptance criteria:** See Table 2. Disregard any peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cytosine <sup>a</sup>	0.12	0.2
Lamivudine-S-sulfoxide <sup>b</sup>	0.19	0.2
Lamivudine-R-sulfoxide <sup>c</sup>	0.21	0.2
Lamivudine-carboxylic acid <sup>d</sup>	0.49	— <sup>e</sup>
Lamivudine diastereomer (Lamivudine-trans) <sup>f</sup>	0.52	— <sup>e</sup>
Lamivudine	0.60	—
Lamivudine-uracil derivative <sup>g</sup>	0.78	0.2
Cyclopropyldiaminopurine abacavir <sup>h</sup>	0.80	0.2
Descyclopropyl abacavir <sup>i</sup>	0.85	0.2
3-Hydroxyabacavir <sup>j</sup>	0.89	— <sup>e</sup>
Abacavir	1.0	— <sup>e</sup>
Any individual unspecified impurity	—	0.2
Total lamivudine related impurities <sup>k</sup>	—	0.6
Total abacavir related impurities <sup>l</sup>	—	1.0

<sup>a</sup> 4-Aminopyrimidin-2(1H)-one (lamivudine related impurity).

<sup>b</sup> 1-[(2R,3S,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine S-oxide.

<sup>c</sup> 1-[(2R,3R,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine S-oxide.

<sup>d</sup> (2R,5S)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.

<sup>e</sup> Process impurity monitored in the drug substance.

<sup>f</sup> 1-[(2S,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

<sup>g</sup> 1-[(2R,5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil.

<sup>h</sup> N<sup>6</sup>-Cyclopropyl-9H-purine-2,6-diamine.

<sup>i</sup> [(1S,4R)-4-(2,6-Diamino-9H-purin-9-yl)cyclopent-2-enyl]methanol.

<sup>j</sup> (1R,2R,4S)-2-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-4-(hydroxymethyl)cyclopentan-1-ol.

<sup>k</sup> Includes all lamivudine related impurities.

<sup>l</sup> Includes all abacavir related and all individual unspecified impurities.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.



# • **USP REFERENCE STANDARDS** (11)

USP Abacavir Sulfate RS

USP Lamivudine RS

USP Lamivudine Resolution Mixture C RS

This is a mixture of lamivudine and the following impurities (other impurities may also be present).

Uracil: Pyrimidine-2,4(1*H*,3*H*)-dione.

$C_4H_4N_2O_2$  112.09

Lamivudine-uracil derivative: 1-[(2*RS*,5*SR*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]uracil.

$C_8H_{10}N_2O_4S$  230.24

Cytosine: 4-Aminopyrimidin-2(1*H*)-one.

$C_4H_5N_3O$  111.10

Lamivudine-*S*-sulfoxide: 1-[(2*R*,3*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.

$C_8H_{11}N_3O_4S$  245.26

Lamivudine-*R*-sulfoxide: 1-[(2*R*,3*R*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine *S*-oxide.

$C_8H_{11}N_3O_4S$  245.26

Lamivudine carboxylic acid: (2*RS*,5*SR*)-5-(Cytosine-1-yl)-1,3-oxathiolane-2-carboxylic acid.

$C_8H_9N_3O_4S$  243.24

Lamivudine diastereomer: 1-[(2*S*,5*S*)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine.

$C_8H_{11}N_3O_3S$  229.26

Salicylic acid: 2-Hydroxybenzoic acid.

$C_7H_6O_3$  138.12

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 5-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection size: 20 μL

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 1.5%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of  $(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$  in the portion of Abacavir Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of abacavir from the Sample solution

$r_S$  = peak area of abacavir from the Standard solution

$C_S$  = concentration of USP Abacavir Sulfate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Abacavir Sulfate in the Sample solution (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

## **IMPURITIES**

### **Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.2%

### **Organic Impurities**

- **PROCEDURE 1: RELATED COMPOUNDS**

Solution A: Trifluoroacetic acid and water (0.05:99.95)

Solution B: Methanol and water (17:3)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	70	30
35	10	90
35.1	95	5
50	95	5

System suitability solution: 0.25 mg/mL of USP Abacavir Related Compounds Mixture RS in water

Sample solution: 0.25 mg/mL of Abacavir Sulfate in water

### **Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 15-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection size: 20 μL

System suitability

Sample: System suitability solution

Suitability requirements

Resolution: NLT 1.5 between abacavir and trans-abacavir

Analysis

Sample: Sample solution

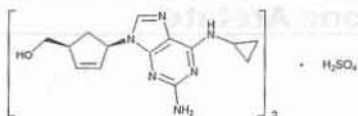
Calculate the percentage of each impurity in the portion of Abacavir Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak area of each impurity from the Sample solution

$r_T$  = sum of the areas of all the peaks from the Sample solution

## **Abacavir Sulfate**



$(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$  670.74

2-Cyclopentene-1-methanol, 4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-, (1*S*-*cis*)-, sulfate (salt) (2:1);

(1*S*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol sulfate (salt) (2:1) [188062-50-2].

## **DEFINITION**

Abacavir Sulfate contains NLT 97.0% and NMT 102.0% of  $(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$ , calculated on the anhydrous and solvent-free basis.

## **IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)

- **B.** The retention time of the major peak of the Sample solution corresponds to that of the System suitability solution, obtained as directed in the test for Organic Impurities, Procedure 2.

- **C. IDENTIFICATION TESTS—GENERAL, Sulfate** (191)

Sample solution: 5 mg/mL

## **ASSAY**

- **PROCEDURE**

Mobile phase: Acetonitrile, phosphoric acid, and water (20:1:180)

Standard solution: 0.04 mg/mL of USP Abacavir Sulfate RS in water

Sample solution: 0.04 mg/mL of Abacavir Sulfate in water

Chromatographic system

(See Chromatography (621), System Suitability.)



## Acceptance criteria

Individual impurities: See Impurity Table 1.

Total impurities: NMT 0.8%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Descyclopropyl abacavir <sup>a</sup>	0.65	0.2
Abacavir	1.00	—
<i>trans</i> -Abacavir <sup>b</sup>	1.04	0.2
O-Pyrimidine derivative abacavir <sup>c</sup>	1.33	0.2
<i>t</i> -Butyl derivative abacavir <sup>d</sup>	1.67	0.2
Any unspecified impurity	—	0.1

<sup>a</sup> [(1*S*,4*R*)-4-(2,6-Diamino-9*H*-purin-9-yl)cyclopent-2-enyl]methanol.<sup>b</sup> [(1*R*,4*R*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-cyclopent-2-enyl]methanol.<sup>c</sup> *N*-(Cyclopropyl-9-[(1*R*,4*S*)-4-[(2,5-diamino-6-chloropyrimidin-4-yl-oxy)methyl]cyclopent-2-enyl]-9*H*-purine-2,6-diamine.<sup>d</sup> 9-[(1*R*,4*S*)-4-(*tert*-Butoxymethyl)cyclopent-2-enyl]-*N*-(cyclopropyl-9*H*-purine-2,6-diamine.

## • PROCEDURE 2: ENANTIOMERIC PURITY

Solution A: Heptane, 2-propanol, and diethylamine (850:150:1).

Solution B: Heptane and 2-propanol (1:1)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	100	0	1.0
25	100	0	1.0
27	0	100	0.8
37	0	100	0.8
39	100	0	1.0
55	100	0	1.0

Diluent: Methanol and trifluoroacetic acid (200:1)

**System suitability solution:** Transfer a quantity of USP Abacavir Stereoisomers Mixture RS to a suitable volumetric flask, add a volume of *Diluent* equivalent to 30% of the final volume, and sonicate until the solid is fully dissolved. Add a volume of 2-propanol equivalent to about 30% of the final volume, mix, and dilute with heptane to volume to obtain 0.4 mg/mL of USP Abacavir Stereoisomers Mixture RS.

**Sample solution:** Transfer 4 mg of Abacavir Sulfate to a 10-mL volumetric flask. Add 3 mL of *Diluent*, and sonicate until the solid is fully dissolved. Add 3 mL of 2-propanol, mix, and dilute with heptane to volume.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 286 nm

Column: 4.6-mm × 25-cm; 10-μm packing L51

Column temperature: 30°

Injection size: 20 μL

**System suitability**

[NOTE—The relative retention times for *trans*-abacavir, abacavir enantiomer, and abacavir are 0.8, 0.9, and 1.0, respectively.]

Sample: System suitability solution

**Suitability requirements**

Resolution: NLT 1.0 between *trans*-abacavir and abacavir enantiomer; NLT 1.5 between abacavir enantiomer and abacavir

**Analysis**

Sample: Sample solution

Calculate the percentage of abacavir enantiomer in the portion of Abacavir Sulfate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak area of abacavir enantiomer from the Sample solution

 $r_T$  = total peak areas of abacavir and abacavir enantiomer from the Sample solution
**Acceptance criteria**

Individual impurities: NMT 0.3% of abacavir enantiomer

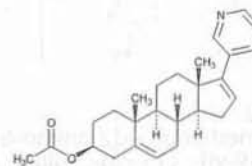
**SPECIFIC TESTS**• **WATER DETERMINATION, Method 1c (921):** NMT 0.5%**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.• **USP REFERENCE STANDARDS (11)**

USP Abacavir Sulfate RS

USP Abacavir Stereoisomers Mixture RS

A mixture of abacavir sulfate, abacavir enantiomer, and *trans*-abacavir.

USP Abacavir Related Compounds Mixture RS

A mixture of abacavir glutarate, O-pyrimidine derivative abacavir, descyclopropyl abacavir, *trans*-abacavir, and *t*-butyl derivative abacavir.**Abiraterone Acetate**

$C_{26}H_{33}NO_2$  391.55  
 Androsta-5,16-dien-3-ol, 17-(3-pyridinyl)-, acetate (ester), (3β);  
 17-(Pyridin-3-yl)androsta-5,16-dien-3β-yl acetate  
 [154229-18-2].

**DEFINITION**

Abiraterone Acetate contains NLT 98.0% and NMT 102.0% of abiraterone acetate ( $C_{26}H_{33}NO_2$ ), calculated on the as-is basis.

**IDENTIFICATION**• **A. INFRARED ABSORPTION (197K)**

• **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

Solution A: 10 mM of ammonium acetate in water  
 Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Acetonitrile (%)	Ethanol (%)
0	50	20	30
40	15	55	30
47	0	20	80
58	0	20	80



Table 1 (Continued)

Time (min)	Solution A (%)	Acetonitrile (%)	Ethanol (%)
60	50	20	30
70	50	20	30

[NOTE—Protect solutions from light.]

**System suitability solution:** 0.625 mg/mL of USP Abiraterone System Suitability Mixture RS in acetonitrile.

[NOTE—See Table 2 for relative retention times of the main components of the mixture.]

Table 2

Name	Relative Retention Time
7-Ketoabiraterone acetate	0.42
$\alpha$ -Epoxyabiraterone acetate	0.62
$\beta$ -Epoxyabiraterone acetate	0.66
Abiraterone	0.69
3-Deoxy-3-acetyl abiraterone-3-ene	0.85
Abiraterone acetate	1.0
Abiraterone ethyl ether	1.18
Abiraterone isopropyl ether	1.26
Anhydro abiraterone	1.29
3-Deoxy 3-chloroabiraterone	1.31
O-Chlorobutylabiraterone	1.33

**Standard solution:** 0.625 mg/mL of USP Abiraterone Acetate RS in acetonitrile

**Sample solution:** 0.625 mg/mL of Abiraterone Acetate in acetonitrile

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3-mm  $\times$  15-cm; 3- $\mu$ m packing L1

**Column temperature:** 15°

**Flow rate:** 0.45 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Samples:** System suitability solution and Standard solution

#### Suitability requirements

**Resolution:** NLT 1.0 between anhydro abiraterone and 3-deoxy 3-chloroabiraterone peaks, System suitability solution

**Relative standard deviation:** NMT 0.73%, Standard solution

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of abiraterone acetate ( $C_{26}H_{33}NO_2$ ) in the portion of Abiraterone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Abiraterone Acetate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Abiraterone Acetate in the Sample solution (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the as-is basis

#### IMPURITIES

• **RESIDUE ON IGNITION (281):** NMT 0.1%

• **ORGANIC IMPURITIES**

[NOTE—Protect solutions from light.]

**Solution A, Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**Sensitivity solution:** 0.3  $\mu$ g/mL of USP Abiraterone Acetate RS in acetonitrile, from Standard solution

#### System suitability

**Samples:** System suitability solution, Standard solution, and Sensitivity solution

#### Suitability requirements

**Resolution:** NLT 1.0 between anhydro abiraterone and 3-deoxy 3-chloroabiraterone peaks, System suitability solution

**Signal-to-noise ratio:** NLT 10, Sensitivity solution

**Relative standard deviation:** NMT 0.73%, Standard solution

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Abiraterone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak area of each impurity from the Sample solution

$r_S$  = peak area of abiraterone acetate from the Standard solution

$C_S$  = concentration of USP Abiraterone Acetate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Abiraterone Acetate in the Sample solution (mg/mL)

$F$  = relative response factor for each individual impurity (see Table 3)

**Acceptance criteria:** See Table 3. Disregard any peak less than 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
$\alpha$ -Epoxyabiraterone acetate	0.62	0.26	0.25
$\beta$ -Epoxyabiraterone acetate	0.66	0.26	0.25
Abiraterone	0.69	1.0	0.20
Abiraterone acetate	1.0	—	—
Abiraterone ethyl ether	1.18	1.0	0.20
Abiraterone isopropyl ether	1.26	1.0	0.20
Unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.80

#### SPECIFIC TESTS

• **WATER DETERMINATION, Method 1c (921):** NMT 0.25%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature and protect from light.

• **USP REFERENCE STANDARDS (11)**

USP Abiraterone Acetate RS

USP Abiraterone System Suitability Mixture RS

It contains Abiraterone Acetate and small amounts of the following:

Abiraterone

17-(Pyridin-3-yl)androsta-5,16-dien-3 $\beta$ -ol.

$C_{24}H_{31}NO$  349.52

Abiraterone ethyl ether

3 $\beta$ -Ethoxy-17-(pyridin-3-yl)androsta-5,16-diene.

$C_{26}H_{35}NO$  377.57

Abiraterone isopropyl ether

3 $\beta$ -Isopropoxy-17-(pyridin-3-yl)androsta-5,16-diene.



$C_{27}H_{37}NO$	391.60
Anhydro abiraterone	
17-(Pyridin-3-yl)androsta-3,5,16-triene.	
$C_{24}H_{29}N$	331.50
O-Chlorobutylabiraterone	
3 $\beta$ -(4-Chlorobutoxy)-17-(pyridin-3-yl)androsta-5,16-diene.	
$C_{28}H_{38}ClNO$	440.07
3-Deoxy-3-acetyl abiraterone-3-ene	
1-[17-(Pyridin-3-yl)androsta-3,5,16-trien-3-yl]ethanone.	
$C_{26}H_{31}NO$	373.53
3-Deoxy 3-chloroabiraterone	
3 $\beta$ -Chloro-17-(pyridin-3-yl)androsta-5,16-diene.	
$C_{24}H_{30}ClN$	367.96
$\alpha$ -Epoxyabiraterone acetate	
17-(Pyridin-3-yl)-16 $\alpha$ ,17 $\alpha$ -epoxyandrost-5-en-3 $\beta$ -yl acetate.	
$C_{26}H_{33}NO_3$	407.55
$\beta$ -Epoxyabiraterone acetate	
17-(Pyridin-3-yl)-16 $\beta$ ,17 $\beta$ -epoxyandrost-5-en-3 $\beta$ -yl acetate.	
$C_{26}H_{33}NO_3$	407.55
7-Ketoabiraterone acetate	
7-Oxo-17-(pyridin-3-yl)androsta-5,16-dien-3 $\beta$ -yl acetate.	
$C_{26}H_{31}NO_3$	405.54

## Abiraterone Acetate Tablets

### DEFINITION

Abiraterone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of abiraterone acetate ( $C_{26}H_{33}NO_2$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Solution A:** 10 mM of ammonium acetate in water  
**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Acetonitrile (%)	Ethanol (%)
0	50	20	30
40	15	55	30
47	0	20	80
58	0	20	80
60	50	20	30
70	50	20	30

[NOTE—Protect solutions from light.]

**System suitability solution:** 0.625 mg/mL of USP Abiraterone System Suitability Mixture RS in acetonitrile.

[NOTE—See Table 2 for relative retention times of the main components of the mixture.]

Table 2

Name	Relative Retention Time
7-Ketoabiraterone acetate	0.42
$\alpha$ -Epoxyabiraterone acetate	0.62
$\beta$ -Epoxyabiraterone acetate	0.66

Table 2 (Continued)

Name	Relative Retention Time
Abiraterone	0.69
3-Deoxy-3-acetyl abiraterone-3-ene	0.85
Abiraterone acetate	1.0
Abiraterone ethyl ether	1.18
Abiraterone isopropyl ether	1.26
Anhydro abiraterone	1.29
3-Deoxy 3-chloroabiraterone	1.31
O-Chlorobutylabiraterone	1.33

**Standard solution:** 0.625 mg/mL of USP Abiraterone Acetate RS in acetonitrile

**Sample solution:** Nominally equivalent to 0.625 mg/mL of abiraterone acetate in acetonitrile, prepared from NLT 20 powdered Tablets as follows. Transfer the powder to a suitable volumetric flask. Add 50% of the flask volume of acetonitrile, shake by mechanical means for 30 min, and dilute with acetonitrile to volume. Pass a portion of the solution through a suitable filter of 0.45- $\mu$ m pore size, and use the clear solution for analysis.

### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm or diode array. [NOTE—Use diode array detector to perform Identification test B.]

**Column:** 3-mm  $\times$  15-cm; 3- $\mu$ m packing L1

**Column temperature:** 15 $^{\circ}$

**Flow rate:** 0.45 mL/min

**Injection volume:** 10  $\mu$ L

### System suitability

**Samples:** System suitability solution and Standard solution

### Suitability requirements

**Resolution:** NLT 1.0 between anhydro abiraterone and 3-deoxy 3-chloroabiraterone peaks, System suitability solution

**Relative standard deviation:** NMT 2.0%, Standard solution

### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of abiraterone acetate ( $C_{26}H_{33}NO_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Abiraterone Acetate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of abiraterone acetate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION <711>

[NOTE—Protect solutions from light.]

**Buffer:** 56.5 mM of monobasic sodium phosphate in water. Adjust with 5 N sodium hydroxide or phosphoric acid to a pH of 4.5.

**Medium:** 0.25% of sodium lauryl sulfate in *Buffer*, 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Standard solution:** 0.3 mg/mL of USP Abiraterone Acetate RS in *Medium* prepared as follows. Transfer USP Abiraterone Acetate RS into a suitable volumetric flask. Add 4% of the flask volume of acetonitrile to dissolve, and dilute with *Medium* to volume.



**Sample solution:** Pass a portion of the solution under test through a suitable filter of 10- $\mu$ m pore size. Use the filtrate.

**Mobile phase:** Acetonitrile, formic acid, and water (55:0.05:45)

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 252 nm

**Column:** 4.6-mm  $\times$  3-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of abiraterone acetate ( $C_{26}H_{33}NO_2$ ) dissolved:

$$(r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 85% (Q) of the labeled amount of abiraterone acetate ( $C_{26}H_{33}NO_2$ ) is dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### ORGANIC IMPURITIES

[NOTE—Protect solutions from light.]

**Solution A, Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Sensitivity solution:** 0.3  $\mu$ g/mL of USP Abiraterone Acetate RS in acetonitrile from *Standard solution*

**System suitability**

**Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution*

**Suitability requirements**

**Resolution:** NLT 1.0 between anhydro abiraterone and 3-deoxy 3-chloroabiraterone peaks, *System suitability solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak area of each impurity from the *Sample solution*

$r_S$  = peak area of abiraterone acetate from the *Standard solution*

$C_S$  = concentration of USP Abiraterone Acetate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of abiraterone acetate in the *Sample solution* (mg/mL)

$F$  = relative response factor for each individual impurity (see *Table 3*)

**Acceptance criteria:** See *Table 3*. Disregard any peak less than 0.05%.

**Table 3**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
7-Ketoabiraterone acetate	0.42	1.4	0.50
$\alpha$ -Epoxyabiraterone acetate	0.62	0.26	0.80
$\beta$ -Epoxyabiraterone acetate	0.66	0.26	0.80
Abiraterone	0.69	1.0	0.40
Abiraterone acetate	1.0	—	—
Abiraterone ethyl ether <sup>a</sup>	1.18	—	—
Abiraterone isopropyl ether <sup>a</sup>	1.26	—	—
Unspecified impurity	—	1.0	0.20
Total impurities	—	—	2.0

<sup>a</sup> This is a process impurity and is controlled in the drug substance monograph. It is included in the table for identification only, and it is not to be reported in the total impurities.

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

### USP REFERENCE STANDARDS (11)

USP Abiraterone Acetate RS

USP Abiraterone System Suitability Mixture RS

It contains Abiraterone Acetate and small amounts of the following:

Abiraterone

17-(Pyridin-3-yl)androsta-5,16-dien-3 $\beta$ -ol.

$C_{24}H_{31}NO$  349.52

Abiraterone ethyl ether

3 $\beta$ -Ethoxy-17-(pyridin-3-yl)androsta-5,16-diene.

$C_{26}H_{35}NO$  377.57

Abiraterone isopropyl ether

3 $\beta$ -Isopropoxy-17-(pyridin-3-yl)androsta-5,16-diene.

$C_{27}H_{37}NO$  391.60

Anhydro abiraterone

17-(Pyridin-3-yl)androsta-3,5,16-triene.

$C_{24}H_{29}N$  331.50

O-Chlorobutylabiraterone

3 $\beta$ -(4-Chlorobutoxy)-17-(pyridin-3-yl)androsta-5,16-diene.

$C_{28}H_{38}ClNO$  440.07

3-Deoxy-3-acetyl abiraterone-3-ene

1-[17-(Pyridin-3-yl)androsta-3,5,16-trien-3-yl]ethanone.

$C_{26}H_{31}NO$  373.53

3-Deoxy 3-chloroabiraterone

3 $\beta$ -Chloro-17-(pyridin-3-yl)androsta-5,16-diene.

$C_{24}H_{30}ClN$  367.96

$\alpha$ -Epoxyabiraterone acetate

17-(Pyridin-3-yl)-16 $\alpha$ ,17 $\alpha$ -epoxyandrost-5-en-3 $\beta$ -yl acetate.

$C_{26}H_{33}NO_3$  407.55

$\beta$ -Epoxyabiraterone acetate

17-(Pyridin-3-yl)-16 $\beta$ ,17 $\beta$ -epoxyandrost-5-en-3 $\beta$ -yl acetate.

$C_{26}H_{33}NO_3$  407.55

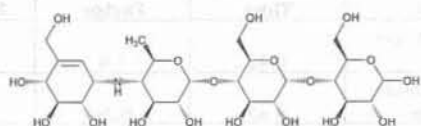
7-Ketoabiraterone acetate

7-Oxo-17-(pyridin-3-yl)androsta-5,16-dien-3 $\beta$ -yl acetate.

$C_{26}H_{31}NO_3$



## Acarbose



$C_{25}H_{43}NO_{18}$  645.60  
 D-Glucose, O-4,6-dideoxy-4-[[[1S-(1 $\alpha$ ,4 $\alpha$ ,5 $\beta$ ,6 $\alpha$ )]-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-;  
 O-4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-glucose [56180-94-0].

### DEFINITION

Acarbose is produced by certain strains of *Actinoplanes utahensis*. It contains NLT 95.0% and NMT 102.0% of acarbose ( $C_{25}H_{43}NO_{18}$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Solution A:** 0.6 mg/mL of monobasic potassium phosphate and 0.35 mg/mL of dibasic sodium phosphate in water

**Mobile phase:** Acetonitrile and *Solution A* (3:1)

**System suitability solution:** 20 mg/mL of USP Acarbose System Suitability Mixture RS in water

**Standard solution:** 20 mg/mL of USP Acarbose RS in water

**Sample solution:** 20 mg/mL of Acarbose in water

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4-mm  $\times$  25-cm; packing L8

**Column temperature:** 35°

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

Identify the acarbose peak and the peaks due to the impurities listed in *Table 1*.

#### Suitability requirements

**Peak-to-valley ratio:** The ratio of the height of the impurity A peak to the height of the valley between the impurity A peak and the acarbose peak is NLT 1.2.

**Chromatogram comparability:** The chromatogram obtained is similar to the chromatogram provided with USP Acarbose System Suitability Mixture RS for the known impurities found.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acarbose ( $C_{25}H_{43}NO_{18}$ ) in the portion of Acarbose taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Acarbose RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–102.0% on the anhydrous basis

### IMPURITIES

#### • RESIDUE ON IGNITION (281)

**Sample:** 1.0 g

Acceptance criteria: NMT 0.2%

#### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-Jan-2018)

#### • CHROMATOGRAPHIC PURITY

**Mobile phase, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Diluted sample solution:** Dilute 1.0 mL of the *Sample solution* with water to 100.0 mL.

#### Analysis

**Samples:** *Sample solution* and *Diluted sample solution*  
 Calculate the percentage of each impurity in the portion of Acarbose taken:

$$\text{Result} = (r_U/r_A) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_A$  = peak response of the main acarbose peak from the *Diluted sample solution*

$F$  = relative response factor for each impurity (see *Table 1*)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity A <sup>a</sup>	0.9	1	0.6
Impurity B <sup>b</sup>	0.8	1.6	0.5
Impurity C <sup>c</sup>	1.2	1	1.5
Impurity D <sup>d</sup>	0.5	1.33	1.0
Impurity E <sup>e</sup>	1.7	0.8	0.2
Impurity F <sup>f</sup>	1.9	0.8	0.3
Impurity G <sup>g</sup>	2.2	0.8	0.3
Impurity H <sup>h</sup>	0.6	1	0.2

<sup>a</sup> O-4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-arabino-hex-2-ulopyranose.

<sup>b</sup> (1R,4R,5S,6R)-4,5,6-Trihydroxy-2-(hydroxymethyl)cyclohex-2-enyl 4-O-[4,6-dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranoside.

<sup>c</sup>  $\alpha$ -D-Glucopyranosyl 4-O-[4,6-dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl]- $\alpha$ -D-glucopyranoside.

<sup>d</sup> 4-O-[4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl]-D-glucopyranose.

<sup>e</sup> O-4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-arabino-hex-2-ulopyranose (4-O- $\alpha$ -acarbosyl-D-fructopyranose).

<sup>f</sup> O-4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose (4-O- $\alpha$ -acarbosyl-D-glucopyranose).

<sup>g</sup>  $\alpha$ -D-Glucopyranosyl O-4,6-dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O- $\alpha$ -D-glucopyranoside ( $\alpha$ -D-glucopyranosyl  $\alpha$ -acarboside).

<sup>h</sup> O-4,6-Dideoxy-4-[[[1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-6-deoxy- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose.



Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual unknown impurity	—	—	0.2
Total impurities	—	—	3.0

<sup>a</sup> O-4,6-Dideoxy-4-[[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)]-O-α-D-glucopyranosyl-(1→4)-D-arabino-hex-2-ulopyranose.

<sup>b</sup> (1R,4R,5S,6R)-4,5,6-Trihydroxy-2-(hydroxymethyl)cyclohex-2-enyl 4-O-[4,6-dideoxy-4-[[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)]-D-glucopyranoside.

<sup>c</sup> α-D-Glucopyranosyl 4-O-[4,6-dideoxy-4-[[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)]-D-glucopyranoside.

<sup>d</sup> 4-O-[4,6-Dideoxy-4-[[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)]-D-glucopyranose.

<sup>e</sup> O-4,6-Dideoxy-4-[[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)]-O-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-arabino-hex-2-ulopyranose (4-O-α-acarbosyl-D-fructopyranose).

<sup>f</sup> O-4,6-Dideoxy-4-[[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)]-O-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1→4)-D-glucopyranose (4-O-α-acarbosyl-D-glucopyranose).

<sup>g</sup> α-D-Glucopyranosyl O-4,6-dideoxy-4-[[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)]-O-α-D-glucopyranosyl-(1→4)-O-α-D-glucopyranoside (α-D-glucopyranosyl α-acarboside).

<sup>h</sup> O-4,6-Dideoxy-4-[[[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-enyl]amino]-α-D-glucopyranosyl-(1→4)]-O-6-deoxy-α-D-glucopyranosyl-(1→4)-D-glucopyranose.

### SPECIFIC TESTS

#### • OPTICAL ROTATION, Specific Rotation (781S)

Sample solution: 10 mg/mL in water

Acceptance criteria: +168° to +183°

#### • pH (791)

Sample solution: 50 mg/mL

Acceptance criteria: 5.5–7.5

#### • WATER DETERMINATION, Method 1c (921): NMT 4.0%

### ADDITIONAL REQUIREMENTS

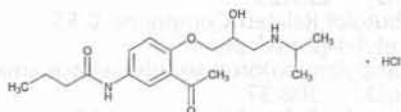
#### • PACKAGING AND STORAGE: Preserve in tight containers.

#### • USP REFERENCE STANDARDS (11)

USP Acarbose RS

USP Acarbose System Suitability Mixture RS

## Acebutolol Hydrochloride



$C_{18}H_{28}N_2O_4 \cdot HCl$  372.89

Butanamide, N-[3-acetyl-4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]-, monohydrochloride, (±); (±)-3'-Acetyl-4'-[2-hydroxy-3-(isopropylamino)propoxy]-butyranilide monohydrochloride [34381-68-5].

### DEFINITION

Acebutolol Hydrochloride contains NLT 98.0% and NMT 102.0% of acebutolol hydrochloride ( $C_{18}H_{28}N_2O_4 \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

• B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• C. **IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements when tested as directed for alkaloidal hydrochlorides.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Methanol, glacial acetic acid, and 0.3% aqueous solution of sodium dodecyl sulfate (675:20:325). Make adjustments if necessary to achieve a retention time for acebutolol of between 4 and 7 min.

**Standard solution:** 0.14 mg/mL of USP Acebutolol Hydrochloride RS in water

**Sample solution:** 0.14 mg/mL of Acebutolol Hydrochloride in water

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 1500 theoretical plates

**Tailing factor:** NMT 2.5

**Relative standard deviation:** 0.73%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acebutolol hydrochloride ( $C_{18}H_{28}N_2O_4 \cdot HCl$ ) in the portion of Acebutolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of acebutolol from the *Sample solution*

$r_S$  = peak response of acebutolol from the *Standard solution*

$C_S$  = concentration of USP Acebutolol Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Acebutolol Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

#### • RESIDUE ON IGNITION (281): NMT 0.1%

#### Delete the following:

#### • HEAVY METALS, Method II (231): NMT 20 ppm (Official 1-Jan-2018)

#### • ORGANIC IMPURITIES

**Solution A:** Mix 2.0 mL of phosphoric acid and 3.0 mL of triethylamine, and dilute with water to 1 L.

**Solution B:** Acetonitrile and *Solution A* (1:1)

**Standard stock solution 1:** 0.2 mg/mL of USP Acebutolol Related Compound A RS prepared as follows. Dissolve a suitable amount of USP Acebutolol Related Compound A RS in a suitable volumetric flask, in 50% of the total volume of acetonitrile, and dilute with *Solution A* to volume.

**Standard stock solution 2:** 0.2 mg/mL of USP Acebutolol Related Compound B RS prepared as follows. Dissolve a suitable amount of USP Acebutolol Related Compound B RS in a suitable volumetric flask, in 50% of the total volume of acetonitrile, and dilute with *Solution A* to volume.



**Standard solution A:** 0.002 mg/mL of USP Acebutolol Hydrochloride RS in *Solution A*

**Standard solution B:** 0.004 mg/mL of USP Acebutolol Related Compound I RS in *Solution A*

**Standard solution C:** 0.002 mg/mL of USP Acebutolol Related Compound A RS in *Solution A* from *Standard stock solution 1*

**Standard solution D:** 0.004 mg/mL of USP Acebutolol Related Compound B RS in *Solution A* from *Standard stock solution 2*

**System suitability solution:** 0.4 µg/mL each of USP Acebutolol Hydrochloride RS and USP Acebutolol Related Compound I RS in *Solution A* from *Standard solution A* and *Standard solution B*

**Sample solution:** 2 mg/mL of Acebutolol Hydrochloride in *Solution A*

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	98	2
2	98	2
30.5	10	90
41	10	90

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.0-mm × 12.5-cm; 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.2 mL/min

**Injection volume:** 25 µL

#### System suitability

**Samples:** *Standard solution A* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 7.0 between acebutolol and acebutolol related compound I, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution A*

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, *Standard solution D*, and *Sample solution*

Calculate the percentage of acebutolol related compound A, acebutolol related compound B, and acebutolol related compound I in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of acebutolol related compound A or acebutolol related compound I from the *Sample solution*

$r_S$  = peak response of acebutolol related compound A or acebutolol related compound I from *Standard solution B*, *Standard solution C*, or *Standard solution D*

$C_S$  = concentration of USP Acebutolol Related Compound A RS or USP Acebutolol Related Compound B RS or USP Acebutolol Related Compound I RS in *Standard solution B*, *Standard solution C*, or *Standard solution D* (mg/mL)

$C_U$  = concentration of Acebutolol Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity from the *Sample solution*

$r_S$  = peak response of acebutolol from *Standard solution A*

$C_S$  = concentration of USP Acebutolol Hydrochloride RS in *Standard solution A* (mg/mL)

$C_U$  = concentration of Acebutolol Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 2*. Disregard peaks below 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acebutolol related compound B <sup>a</sup>	0.72	0.2
Acebutolol related compound I <sup>b</sup>	0.91	0.2
Acebutolol	1.00	—
Acebutolol related compound A <sup>c</sup>	1.48	0.1
Any unspecified impurity	—	0.10
Total impurities <sup>d</sup>	—	0.5

<sup>a</sup> N-[3-Acetyl-4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl]acetamide.

<sup>b</sup> N-[3-Acetyl-4-[3-(ethylamino)-2-hydroxypropoxy]phenyl]butyramide.

<sup>c</sup> N-(3-Acetyl-4-hydroxyphenyl)butyramide.

<sup>d</sup> Total impurities include specified and unspecified impurities.

#### SPECIFIC TESTS

##### • pH (791)

**Sample:** 10 mg/mL of Acebutolol Hydrochloride in water

**Acceptance criteria:** 4.5–7.0

##### • LOSS ON DRYING (731)

**Analysis:** Dry at 105° for 3 h.

**Acceptance criteria:** NMT 1.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

##### • USP REFERENCE STANDARDS (11)

USP Acebutolol Hydrochloride RS

USP Acebutolol Related Compound A RS

N-(3-Acetyl-4-hydroxyphenyl)butyramide.

C<sub>12</sub>H<sub>15</sub>NO<sub>3</sub> 221.25

USP Acebutolol Related Compound B RS

N-[3-Acetyl-4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl]acetamide.

C<sub>16</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub> 308.37

USP Acebutolol Related Compound I RS

N-[3-Acetyl-4-[3-(ethylamino)-2-hydroxypropoxy]phenyl]butyramide.

C<sub>17</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub> 322.40

## Acebutolol Hydrochloride Capsules

#### DEFINITION

Acebutolol Hydrochloride Capsules contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of acebutolol (C<sub>18</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub>).



**IDENTIFICATION****Add the following:**

- ▲ **A.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*. ▲<sup>USP40</sup>

**Change to read:**

- ▲**B.**▲<sup>USP40</sup> The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****Change to read:**• **PROCEDURE**

**Buffer:** Dissolve 2.4 g of sodium 1-decanesulfonate in 1000 mL of water. Adjust with glacial acetic acid to a pH of 3.5.

**Mobile phase:** Methanol and *Buffer* (60:40)

**Standard solution:** 0.22 mg/mL of USP Acebutolol Hydrochloride RS in methanol. [NOTE—This is equivalent to 0.2 mg/mL of acebutolol.]

**Sample stock solution:** Nominally 1 mg/mL of acebutolol prepared as follows. Transfer an equivalent to 200 mg of acebutolol, from the contents of NLT 20 Capsules, to a 200-mL volumetric flask. Add 180 mL of methanol, and stir by mechanical means for 30 min. Dilute with methanol to volume.

**Sample solution:** Nominally 0.2 mg/mL of acebutolol in methanol from *Sample stock solution*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm. ▲For *Identification A*, use a diode-array detector in the range of 200–400 nm. ▲<sup>USP40</sup>

**Column:** 3.9-mm × 15-cm; ▲4-μm▲<sup>USP40</sup> packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acebutolol ( $C_{18}H_{28}N_2O_4$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of acebutolol from the *Sample solution*

$r_S$  = peak response of acebutolol from the *Standard solution*

$C_S$  = concentration of USP Acebutolol Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acebutolol from the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of acebutolol, ▲336.43▲<sup>USP40</sup>

$M_{r2}$  = molecular weight of acebutolol hydrochloride, 372.89

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS****Change to read:**• **DISSOLUTION** (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Standard solution:** A known concentration of USP Acebutolol Hydrochloride RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV

**Analytical wavelength:** 232 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

▲Calculate the percentage of the labeled amount of acebutolol ( $C_{18}H_{28}N_2O_4$ ) dissolved:

$$(A_U/A_S) \times C_S \times V \times (1/L) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of acebutolol in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

$M_{r1}$  = molecular weight of acebutolol, 336.43

$M_{r2}$  = molecular weight of acebutolol hydrochloride, 372.89▲<sup>USP40</sup>

**Tolerances:** NLT 80% (Q) of the labeled amount of acebutolol ( $C_{18}H_{28}N_2O_4$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**IMPURITIES****Change to read:**• **ORGANIC IMPURITIES**

**Test 1**

**Buffer:** Prepare as directed in the *Assay*.

**Mobile phase:** Methanol and *Buffer* (44:56)

**Diluent:** Methanol and *Buffer* (50:50)

**Standard stock solution:** 0.6 mg/mL of USP

Acebutolol Hydrochloride RS prepared as follows. To a suitable amount of USP Acebutolol Hydrochloride RS in a suitable volumetric flask, add methanol, about 24% of the flask volume, swirl to dissolve, and dilute with *Diluent* to volume.

**Standard solution:** 1.4 μg/mL of USP Acebutolol Hydrochloride RS in *Diluent* from *Standard stock solution*

**Sample stock solution:** Nominally 2.5 mg/mL of acebutolol prepared as follows. Transfer a portion of the contents of 20 opened Capsules, equivalent to 250 mg of acebutolol, to a 100-mL volumetric flask. Add 25 mL of methanol and shake by mechanical means for 15 min. Dilute with *Diluent* to volume. Centrifuge a portion of this solution and use the supernatant.

**Sample solution:** Nominally 250 μg/mL of acebutolol in *Diluent* from *Sample stock solution*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 240 nm  
 Column: 3.9-mm × 15-cm; 4-μm packing L1  
 Flow rate: 1 mL/min  
 Injection volume: 35 μL  
 Run time: NLT 2 times the retention time of acebutolol

**System suitability**

Sample: Standard solution

**Suitability requirements**

Relative standard deviation: NMT 6.0%

**Analysis**

Samples: Diluent, Standard solution, and Sample solution

Calculate the percentage of each impurity eluting before the acebutolol peak in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $r_U$  = peak response of each individual impurity from the *Sample solution*  
 $r_S$  = peak response of acebutolol from the *Standard solution*  
 $C_S$  = concentration of USP Acebutolol Hydrochloride RS in the *Standard solution* (μg/mL)  
 $C_U$  = nominal concentration of acebutolol in the *Sample solution* (μg/mL)  
 $M_{r1}$  = molecular weight of acebutolol, 336.43<sup>▲</sup><sub>USP40</sub>  
 $M_{r2}$  = molecular weight of acebutolol hydrochloride, 372.89

**Acceptance criteria:** NMT 0.5% of any individual impurity. Disregard any peaks from the *Diluent*.

**Test 2**

**Buffer and System suitability:** Proceed as directed in *Test 1*.

**Mobile phase:** Methanol and *Buffer* (50:50)

**Standard stock solution:** 0.6 mg/mL of USP

Acebutolol Hydrochloride RS prepared as follows. To a suitable amount of USP Acebutolol Hydrochloride RS in a suitable volumetric flask, add methanol, about 24% of the flask volume, swirl to dissolve, and dilute with *Mobile phase* to volume.

**Standard solution:** 1.4 μg/mL of USP Acebutolol Hydrochloride RS in *Mobile phase* from *Standard stock solution*

**Sample stock solution:** Nominally 2.5 mg/mL of acebutolol prepared as follows. Transfer a portion of the contents of 20 opened Capsules, equivalent to 250 mg of acebutolol, to a 100-mL volumetric flask. Add 25 mL of methanol and shake by mechanical means for 15 min. Dilute with *Mobile phase* to volume.

**Sample solution:** Nominally 250 μg/mL of acebutolol in *Diluent* from *Sample stock solution* prepared as follows. Centrifuge a portion of *Sample stock solution*, and transfer 10.0 mL of the clear supernatant to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.) Proceed as directed in *Test 1* except for the *Injection volume* and *Run time*.

**Injection volume:** 70 μL

**Run time:** NLT 3 times the retention time of acebutolol

**Analysis**

Samples: *Mobile phase*, *Standard solution*, and *Sample solution*

Calculate the percentage of each impurity eluting after the acebutolol peak in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $r_U$  = peak response of each individual impurity from the *Sample solution*  
 $r_S$  = peak response of acebutolol from the *Standard solution*  
 $C_S$  = concentration of USP Acebutolol Hydrochloride RS in the *Standard solution* (μg/mL)  
 $C_U$  = nominal concentration of acebutolol from the *Sample solution* (μg/mL)  
 $M_{r1}$  = molecular weight of acebutolol, 336.43<sup>▲</sup><sub>USP40</sub>  
 $M_{r2}$  = molecular weight of acebutolol hydrochloride, 372.89

**Acceptance criteria**

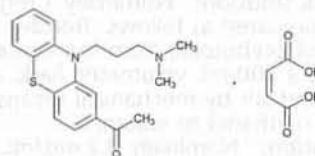
**Test 2:** NMT 0.5% of any individual impurity. Disregard any peaks from the *Mobile phase*.

**Sum of impurities from Test 1 and Test 2:** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
 USP Acebutolol Hydrochloride RS

## Acepromazine Maleate



$C_{19}H_{22}N_2O_5 \cdot C_4H_4O_4$  442.53  
 Ethanone, 1-[10-[3-(dimethylamino)propyl]-10H-phenothiazin-2-yl]-, (Z)-2-butenedioate (1:1);  
 10-[3-(Dimethylamino)propyl]phenothiazin-2-yl methyl ketone maleate (1:1) [3598-37-6].

**DEFINITION**

Acepromazine Maleate contains NLT 98.0% and NMT 101.0% of acepromazine maleate ( $C_{19}H_{22}N_2O_5 \cdot C_4H_4O_4$ ), calculated on the anhydrous basis.

Throughout the following procedures, protect samples, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak for acepromazine of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE**

**Buffer:** Add 6 mL of triethylamine to 700 mL of water, and adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** Acetonitrile and *Buffer* (300:700)

**Standard stock solution:** 1 mg/mL of USP

Acepromazine Maleate RS in 0.05 N hydrochloric acid  
**Standard solution:** 0.1 mg/mL of USP Acepromazine Maleate RS in water from *Standard stock solution*

**Sample stock solution:** 1 mg/mL of Acepromazine Maleate in 0.05 N hydrochloric acid

**Sample solution:** 0.1 mg/mL of Acepromazine Maleate in water from *Sample stock solution*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 280 nm

Column: 4-mm × 15-cm; 5-μm packing L7

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acepromazine maleate ( $C_{19}H_{22}N_2OS \cdot C_4H_4O_4$ ) in the portion of Acepromazine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area response from the *Sample solution*

$r_S$  = peak area response from the *Standard solution*

$C_S$  = concentration of USP Acepromazine Maleate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–101.0% on the anhydrous basis

## IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.2%

• **ORGANIC IMPURITIES**

Conduct this test without exposure to daylight, and with the minimum necessary exposure to artificial light.

Diluent: Methanol and diethylamine (19:1)

Sample solution: 20.0 mg/mL of Acepromazine Maleate in Diluent

Standard solution: 0.1 mg/mL of Acepromazine Maleate in Diluent from the *Sample solution*

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μL

Developing solvent system: *n*-Heptane, isobutyl alcohol, and diethylamine (75:17:8)

Analysis

Samples: *Standard solution* and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths the length of the plate. Remove the plate from the chamber and allow to air dry. Examine the plate under short-wavelength UV light.

Acceptance criteria: 0.5%; no spot, other than the principal acepromazine spot and any at the origin, observed in the chromatogram of the *Sample solution* is more intense than the principal spot observed in the chromatogram of the *Standard solution*.

## SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** (741): 136°–139°

• **PH** (791)

Sample solution: 10 mg/mL of Acepromazine Maleate in water

Acceptance criteria: 4.0–5.5

• **WATER DETERMINATION, Method I** (921): NMT 1.0%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.

• **LABELING:** Label it to indicate that it is for veterinary use only.

## • USP REFERENCE STANDARDS (11)

USP Acepromazine Maleate RS

## Acepromazine Maleate Injection

### DEFINITION

Acepromazine Maleate Injection is a sterile solution of Acepromazine Maleate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of acepromazine maleate ( $C_{19}H_{22}N_2OS \cdot C_4H_4O_4$ ).

Throughout the following procedures, protect samples, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

### IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

Sample: To a volume of Injection, equivalent to 20 mg of acepromazine maleate, add 2 mL of water and 3 mL of 2 N sodium hydroxide, and extract with two 5-mL portions of cyclohexane. Combine the cyclohexane extracts, and evaporate to dryness under vacuum, using gentle heat if necessary.

Acceptance criteria: Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

• **PROCEDURE**

Buffer: Add 6 mL of triethylamine to 700 mL of water, and adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Acetonitrile and Buffer (300:700)

Standard stock solution: 1 mg/mL of USP

Acepromazine Maleate RS in 0.05 N hydrochloric acid

Standard solution: 0.1 mg/mL of USP Acepromazine Maleate RS in water from *Standard stock solution*

Sample stock solution: 1 mg/mL of Acepromazine Maleate in 0.05 N hydrochloric acid from an appropriately diluted volume of Injection

Sample solution: Nominally 0.1 mg/mL of Acepromazine Maleate in water from *Sample stock solution*

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 280 nm

Column: 4-mm × 15-cm; 5-μm packing L7

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acepromazine maleate ( $C_{19}H_{22}N_2OS \cdot C_4H_4O_4$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Acepromazine Maleate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)



Acceptance criteria: 90.0%–110.0%

### SPECIFIC TESTS

- **pH (791):** 4.5–5.8
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 4.5 USP Endotoxin Units/mg of acepromazine maleate
- **STERILITY TESTS (71):** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products (1)*.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant, single-dose or multiple-dose containers as described in *Packaging and Storage Requirements (659)*, *Injection Packaging*. Store at controlled room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS (11)**  
USP Acepromazine Maleate RS  
USP Endotoxin RS

## Acepromazine Maleate Tablets

### DEFINITION

Acepromazine Maleate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acepromazine maleate ( $C_{19}H_{22}N_2O_5 \cdot C_4H_4O_4$ ).

Throughout the following procedures, protect samples, the USP Reference Standard, and solutions containing them, by conducting the procedures without delay, under subdued light, or using low-actinic glassware.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**  
**Sample:** To a quantity of powdered Tablets, equivalent to 20 mg of acepromazine maleate, add 2 mL of water and 3 mL of 2 N sodium hydroxide, and extract with two 5-mL portions of cyclohexane. Combine the cyclohexane extracts, and evaporate to dryness under vacuum, using gentle heat if necessary.  
**Acceptance criteria:** Meet the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

- **PROCEDURE**  
**Buffer:** Add 6 mL of triethylamine to 700 mL of water, and adjust with phosphoric acid to a pH of 2.5.  
**Mobile phase:** Acetonitrile and Buffer (300:700)  
**Standard stock solution:** 1 mg/mL of USP Acepromazine Maleate RS in 0.05 N hydrochloric acid  
**Standard solution:** 0.1 mg/mL of USP Acepromazine Maleate RS in water from *Standard stock solution*  
**Sample stock solution:** Transfer NLT 10 Tablets to a 200-mL volumetric flask, add 100 mL of 0.05 N hydrochloric acid, and sonicate for 10 min. Shake by mechanical means for 30 min, and dilute with 0.05 N hydrochloric acid to volume.  
**Sample solution:** Nominally 0.1 mg/mL of Acepromazine Maleate in water from *Sample stock solution*. Pass a portion of this solution through a filter of 0.5- $\mu$ m or finer pore size.

### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4-mm  $\times$  15-cm; 5- $\mu$ m packing L7

Flow rate: 1 mL/min

Injection volume: 10  $\mu$ L

### System suitability

Sample: *Standard solution*

### Suitability requirements

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acepromazine maleate ( $C_{19}H_{22}N_2O_5 \cdot C_4H_4O_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Acepromazine Maleate RS in the *Standard solution* (mg/mL)

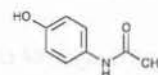
$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** Label the Tablets to indicate that they are for veterinary use only.
- **USP REFERENCE STANDARDS (11)**  
USP Acepromazine Maleate RS

## Acetaminophen



$C_8H_9NO_2$

151.16

Acetamide, N-(4-hydroxyphenyl)-;  
4'-Hydroxyacetanilide [103-90-2].

### DEFINITION

Acetaminophen contains NLT 98.0% and NMT 102.0% of acetaminophen ( $C_8H_9NO_2$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

- **PROCEDURE**  
Use low-actinic glassware for preparation of the *Sample solution*.  
**Solution A:** 1.7 g/L of monobasic potassium phosphate and 1.8 g/L of dibasic sodium phosphate, anhydrous  
**Solution B:** Methanol  
**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	99	1
3.0	99	1
7.0	19	81



Table 1 (Continued)

Time (min)	Solution A (%)	Solution B (%)
7.1	99	1
10.0	99	1

**Standard solution:** 0.1 mg/mL of USP Acetaminophen RS in methanol

**Sample solution:** 0.1 mg/mL of Acetaminophen in methanol

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 10-cm; 3.5-μm packing L7

**Column temperature:** 35°

**Flow rate:** 1.0 mL/min

**Injection volume:** 5 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acetaminophen ( $C_6H_9NO_2$ ) in the portion of Acetaminophen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Acetaminophen in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**Delete the following:**

- **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1: Jan-2018)

- **LIMIT OF FREE 4-AMINOPHENOL**

**Solution A, Solution B, Mobile phase, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 1.25 μg/mL of USP 4-Aminophenol RS in methanol

**Sample solution:** 25 mg/mL of Acetaminophen in methanol

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for 4-aminophenol and acetaminophen are 0.6 and 1.0, respectively.]

**Suitability requirements**

**Relative standard deviation:** NMT 5.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 4-aminophenol in the portion of Acetaminophen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of 4-aminophenol from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP 4-Aminophenol RS in the *Standard solution* (μg/mL)

$C_U$  = concentration of Acetaminophen in the *Sample solution* (μg/mL)

**Acceptance criteria:** NMT 0.005%

• **ORGANIC IMPURITIES**

**Solution A:** Methanol, water, glacial acetic acid (50:950:1)

**Solution B:** Methanol, water, glacial acetic acid (500:500:1)

**Mobile phase:** See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	82	18
8	82	18
53	0	100
58	0	100
59	82	18
73	82	18

**Diluent:** Methanol

**System suitability solution:** 20 μg/mL of USP Acetaminophen RS and 80 μg/mL each of USP Acetaminophen Related Compound B RS and USP Acetaminophen Related Compound C RS in *Diluent*

**Standard solution:** 1.25 μg/mL of USP Acetaminophen Related Compound D RS and 0.25 μg/mL of USP Acetaminophen Related Compound J RS in *Diluent*

**Sample solution:** 25 mg/mL of Acetaminophen in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Flow rate:** 0.9 mL/min

**Column temperature:** 40°

**Injection volume:** 5 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 3* for relative retention time values.]

**Suitability requirements**

**Tailing factor:** NMT 2.0 for acetaminophen related compound D, *Standard solution*

**Resolution:** NLT 2.0 between acetaminophen and acetaminophen related compound B; NLT 1.5 between acetaminophen related compound B and acetaminophen related compound C, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for acetaminophen related compound D, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acetaminophen related compound J in the portion of Acetaminophen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of acetaminophen related compound J from the *Sample solution*

$r_S$  = peak response of acetaminophen related compound J from the *Standard solution*

$C_S$  = concentration of USP Acetaminophen Related Compound J RS in the *Standard solution* (μg/mL)

$C_U$  = concentration of Acetaminophen in the *Sample solution* (μg/mL)

Calculate the percentage of acetaminophen related compounds B, C, and D and any unspecified impurity in the portion of Acetaminophen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$



- $r_U$  = peak response of each specified or unspecified impurity from the *Sample solution*  
 $r_S$  = peak response of acetaminophen related compound D from the *Standard solution*  
 $C_S$  = concentration of USP Acetaminophen Related Compound D RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of Acetaminophen in the *Sample solution* ( $\mu\text{g/mL}$ )  
 $F$  = relative response factor for each impurity shown in Table 3

**Acceptance criteria:** See Table 3. [NOTE—The relative retention times and relative response factors in Table 3 (where applicable) are calculated relative to those of acetaminophen related compound D.]

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Acetaminophen	0.43	—	—
Acetaminophen related compound B <sup>a</sup>	0.67	1.2	0.05
Acetaminophen related compound C <sup>b</sup>	0.71	0.38	0.05
Acetaminophen related compound D <sup>c</sup>	1.0	1.0	0.05
Acetaminophen related compound J <sup>d</sup>	1.73	—	0.001
Individual unspecified impurity	—	1.0	0.05
Total impurities	—	—	0.1

<sup>a</sup> N-(4-Hydroxyphenyl)propanamide.

<sup>b</sup> N-(2-Hydroxyphenyl)acetamide.

<sup>c</sup> N-Phenylacetamide.

<sup>d</sup> N-(4-Chlorophenyl)acetamide (p-chloroacetanilide).

### SPECIFIC TESTS

#### • LOSS ON DRYING (731)

**Analysis:** Dry at 105° to constant weight.

**Acceptance criteria:** NMT 0.5%

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature. Protect from moisture and heat.

#### • USP REFERENCE STANDARDS (11)

USP Acetaminophen RS

USP Acetaminophen Related Compound B RS

N-(4-Hydroxyphenyl)propanamide.

$\text{C}_9\text{H}_{11}\text{NO}_2$  165.19

USP Acetaminophen Related Compound C RS

N-(2-Hydroxyphenyl)acetamide.

$\text{C}_8\text{H}_9\text{NO}_2$  151.16

USP Acetaminophen Related Compound D RS

N-Phenylacetamide.

$\text{C}_8\text{H}_9\text{NO}$  135.17

USP Acetaminophen Related Compound J RS

N-(4-Chlorophenyl)acetamide (p-chloroacetanilide).

$\text{C}_8\text{H}_8\text{ClNO}$  169.61

USP 4-Aminophenol RS

$\text{C}_6\text{H}_7\text{NO}$  109.13

## Acetaminophen Capsules

### DEFINITION

Acetaminophen Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ).

### IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Sample solution:** 1 mg/mL of acetaminophen prepared as follows. Triturate from contents of the Capsules in methanol. Filter, and use the clear filtrate.

**Chromatographic system**

**Developing solvent system:** Methylene chloride and methanol (4:1)

**Acceptance criteria:** Meet the requirements

### ASSAY

#### • PROCEDURE

**Mobile phase:** Methanol and water (1:3)

**Standard solution:** 0.01 mg/mL of USP Acetaminophen RS in *Mobile phase*

**Sample stock solution:** Weigh the contents of NLT 20 Capsules, and calculate the average weight of the contents of each Capsule. Mix the combined contents of the Capsules, and transfer a portion, equivalent to 100 mg of acetaminophen, to a 200-mL volumetric flask. Add 100 mL of *Mobile phase*, shake by mechanical means for 10 min, and dilute with *Mobile phase* to volume. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass a portion of this solution through a filter of 0.5- $\mu\text{m}$  or finer pore size, discarding the first 10 mL of the filtrate.

**Sample solution:** Nominally 0.01 mg/mL of acetaminophen from the *Sample stock solution* in *Mobile phase*. Pass a portion of this solution through a filter of 0.5- $\mu\text{m}$  or finer pore size, discarding the first 10 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 243 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu\text{L}$

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)



Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Standard solution: A known concentration of USP Acetaminophen RS in Medium

Sample solution: A filtered portion of the solution under test, suitably diluted with Medium to obtain a concentration similar to that of the Standard solution

### Instrumental conditions

Mode: UV

Analytical wavelength: 249 nm

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

## IMPURITIES

### • 4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227): Meet the requirements

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in tight containers, and store at controlled room temperature.

### • USP REFERENCE STANDARDS (11)

USP Acetaminophen RS

## Acetaminophen Oral Solution

## DEFINITION

Acetaminophen Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ).

## IDENTIFICATION

### • A. The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

### • B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Sample solution: Nominally 1 mg/mL of acetaminophen in methanol from the Oral Solution

Chromatographic system

Developing solvent system: Methylene chloride and methanol (4:1)

Acceptance criteria: Meets the requirements

## ASSAY

### • PROCEDURE

Mobile phase: Methanol and water (1:3)

Standard solution: 0.01 mg/mL of USP Acetaminophen RS in Mobile phase

Sample stock solution: Nominally 2 mg/mL in Mobile phase, prepared as follows. Transfer 500 mg of acetaminophen from a measured volume of Oral Solution to a 250-mL volumetric flask, and dilute with Mobile phase to volume.

Sample solution: Nominally 0.01 mg/mL of acetaminophen in Mobile phase from the Sample stock solution. Pass a portion of this solution through a filter of 0.5- $\mu$ m or finer pore size, discarding the first 10 mL of the filtrate. Use the clear filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 243 nm

Column: 3.9-mm  $\times$  30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10  $\mu$ L

### System suitability

Sample: Standard solution

### Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Acetaminophen RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of acetaminophen in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DELIVERABLE VOLUME (698): Meets the requirements for oral solutions packaged in multiple-unit containers

### • UNIFORMITY OF DOSAGE UNITS (905): Meets the requirements for oral solutions packaged in single-unit containers

## IMPURITIES

### • 4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227): Meets the requirements

## SPECIFIC TESTS

### • pH (791): 3.8–6.1

### • ALCOHOL DETERMINATION, Method II (611) (if present)

Analysis: Determine by the gas-liquid chromatographic procedure, using acetone as the internal standard.

Acceptance criteria: 90.0%–115.0% of the labeled amount of alcohol ( $C_2H_5OH$ )

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in tight containers, and store at controlled room temperature.

### • USP REFERENCE STANDARDS (11)

USP Acetaminophen RS

## Acetaminophen for Effervescent Oral Solution

## DEFINITION

Acetaminophen for Effervescent Oral Solution contains, in each 100 g, NLT 5.63 g and NMT 6.88 g of acetaminophen ( $C_8H_9NO_2$ ).

## IDENTIFICATION

### • A. A 10-g portion dissolves, with effervescence, in water when dissolved as directed for the Sample solution in the Assay.

### • B. The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

### • C. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Sample solution: Triturate 0.4 g of the powder with 25 mL of methanol, and filter.



**Chromatographic system**

Developing solvent system: Methylene chloride and methanol (4:1)

Acceptance criteria: Meets the requirements

**ASSAY****• PROCEDURE**

**Mobile phase:** Methanol and water (1:3)

**Standard solution:** 0.01 mg/mL of USP Acetaminophen RS in *Mobile phase*

**Sample stock solution:** Dissolve 10 g of Acetaminophen for Effervescent Oral Solution in 200 mL of water in a 1000-mL volumetric flask, using gentle heat if necessary, until effervescence subsides, and then dilute with water to volume.

**Sample solution:** Nominally 0.16 mg/mL of acetaminophen in *Mobile phase* from the *Sample stock solution*. Pass a portion of this solution through a filter of 0.5- $\mu$ m or finer pore size, discarding the first 10 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 243 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in g, of acetaminophen ( $C_8H_9NO_2$ ) in 100 g of Acetaminophen for Effervescent Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F_1) \times L \times F_2$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

$F_1$  = conversion factor, 1000 mg/g

$L$  = label claim (mg/unit)

$F_2$  = conversion factor, 100, based on the label claim of g of acetaminophen per 100 g of sample

Acceptance criteria: 5.63–6.88 g

**PERFORMANCE TESTS**

**• MINIMUM FILL (755):** Meets the requirements for solids packaged in multiple-unit containers

**• UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for solids packaged in single-unit containers

**IMPURITIES**

**• 4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227):** Meets the requirements

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in air-tight containers, and store at controlled room temperature.

- USP REFERENCE STANDARDS (11)**  
USP Acetaminophen RS

**Acetaminophen Suppositories****DEFINITION**

Acetaminophen Suppositories contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Sample solution:** Transfer the equivalent of 20 mg of acetaminophen from a portion of Suppositories to a beaker. Add 20 mL of methanol and heat on a steam bath until melted. Remove the beaker from the steam bath, allow to cool with occasional stirring, and filter. Use the clear filtrate.

**Chromatographic system**

Developing solvent system: Methylene chloride and methanol (4:1)

Acceptance criteria: Meet the requirements

**ASSAY****• PROCEDURE**

**Mobile phase:** Methanol and water (1:3)

**Standard solution:** 0.01 mg/mL of USP Acetaminophen RS in *Mobile phase*

**Sample stock solution:** Nominally 0.5 mg/mL of acetaminophen prepared as follows. Tare a small dish and a glass rod, place NLT 5 Suppositories in the dish, heat gently on a steam bath until melted, stir, cool while stirring, and weigh. Transfer a weighed portion of the mass, equivalent to 100 mg of acetaminophen, to a separator, add 30 mL of solvent hexane, and dissolve. Add 30 mL of water, shake gently, and allow the phases to separate. If an emulsion forms, allow sufficient time for it to separate. Transfer the aqueous layer to a 200-mL volumetric flask, and wash the solvent hexane in the separator with three 30-mL portions of water, adding the washings to the volumetric flask. Dilute with *Mobile phase* to volume.

**Sample solution:** Nominally 0.01 mg/mL of acetaminophen in *Mobile phase* from the *Sample stock solution*. Pass a portion of this solution through a filter of 0.5- $\mu$ m or finer pore size, discarding the first 10 mL of the filtrate. Use the clear filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 243 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Suppositories taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*



- $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

### IMPURITIES

#### • 4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227)

**Buffer:** 4.0 g/L of sodium citrate dihydrate and 1.5 g/L of anhydrous citric acid, in water

**Diluent:** *Buffer* and acetonitrile (9:1)

**Sample stock solution:** Approximately 12–13 mg/mL of acetaminophen prepared as follows. Transfer an appropriate number of whole Suppositories to a suitable volumetric flask. Add *Diluent* until the flask is about half filled and sonicate for 1 h with frequent swirling. Allow to cool and then dilute with *Diluent* to volume.

**Sample solution:** Approximately 4.8–5.2 mg/mL of acetaminophen in *Diluent* from the *Sample stock solution* prepared as follows. Pipet 20.0 mL of the *Sample stock solution* into a 50-mL volumetric flask and dilute with *Diluent* to volume.

**Standard stock solution:** Prepare as indicated in the chapter.

**Standard solution:** Add 20.0 mL of the *Sample stock solution* and 15.0 mL of the *Standard stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

Acceptance criteria: Meet the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature or in a cool place.
- **USP REFERENCE STANDARDS (11)**  
USP Acetaminophen RS

## Acetaminophen Oral Suspension

### DEFINITION

Acetaminophen Oral Suspension is a suspension of Acetaminophen in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Sample:** Transfer a volume of Oral Suspension, equivalent to 240 mg of acetaminophen, to a separator. Add 50 mL of ethyl acetate, and shake. Filter the ethyl acetate extract through a funnel containing glass wool and 10 g of anhydrous sodium sulfate. Collect the filtrate in a beaker, and evaporate on a steam bath to dryness. Dry the residue under vacuum over silica gel.

Acceptance criteria: The crystals so obtained meet the requirements.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Methanol and water (1:3)

**Standard solution:** 0.01 mg/mL of USP Acetaminophen RS in *Mobile phase*

**Sample stock solution:** Nominally 0.5 mg/mL of acetaminophen prepared as follows. Transfer 100 mg of acetaminophen from a volume of Oral Suspension, previously well shaken, to a 200-mL volumetric flask. Add 100 mL of *Mobile phase*, and shake by mechanical means for 10 min. Dilute with *Mobile phase* to volume.

**Sample solution:** Nominally 0.01 mg/mL of acetaminophen from the *Sample stock solution* in *Mobile phase*.

Pass a portion of this solution through a filter of 0.5- $\mu$ m pore size or finer, discarding the first 10 mL of the filtrate. Use the clear filtrate.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 243 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu$ L

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for oral suspensions packaged in single-unit containers
- **DELIVERABLE VOLUME (698):** Meets the requirements for oral suspensions packaged in multiple-unit containers

### IMPURITIES

- **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227):** Meets the requirements

### SPECIFIC TESTS

- **pH (791):** 4.0–6.9

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Acetaminophen RS  
USP 4-Aminophenol RS

## Acetaminophen Tablets

### DEFINITION

Acetaminophen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Sample solution:** Nominally 1 mg/mL of acetaminophen prepared as follows. Triturate 50 mg of acetaminophen from powdered Tablets in 50 mL of methanol, and filter. Use the clear filtrate.

### Chromatographic system

**Developing solvent system:** Methylene chloride and methanol (4:1)



Acceptance criteria: Meet the requirements

## ASSAY

### PROCEDURE

Mobile phase: Methanol and water (1:3)

Standard solution: 0.01 mg/mL of USP Acetaminophen RS in Mobile phase

Sample stock solution: Nominally 0.5 mg/mL of acetaminophen prepared as follows. Weigh, and finely powder NLT 20 Tablets. Transfer 100 mg of acetaminophen from a portion of powdered Tablets to a 200-mL volumetric flask, add 100 mL of Mobile phase, shake by mechanical means for 10 min, sonicate for 5 min, and dilute with Mobile phase to volume.

Sample solution: Nominally 0.01 mg/mL of acetaminophen in Mobile phase from the Sample stock solution. Pass a portion of this solution through a filter of 0.5- $\mu$ m or finer pore size, discarding the first 10 mL of the filtrate. Use the clear filtrate.

### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 243 nm

Column: 3.9-mm  $\times$  30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10  $\mu$ L

### System suitability

Sample: Standard solution

### Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 2

Relative standard deviation: NMT 2.0%

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Acetaminophen RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of acetaminophen in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### DISSOLUTION (711)

Medium: pH 5.8 phosphate buffer (see Reagents, Indicators, and Solutions—Buffer Solutions); 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: A known concentration of USP Acetaminophen RS in Medium

Sample solution: A filtered portion of the solution under test, suitability diluted with Medium to obtain a concentration similar to that of the Standard solution

### Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 243 nm

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) is dissolved.

### For Tablets labeled as chewable

Medium: pH 5.8 phosphate buffer (see Reagents, Indicators, and Solutions—Buffer Solutions); 900 mL

Apparatus 2: 75 rpm

Time: 45 min

Standard solution, Sample solution, Instrumental conditions, and Analysis: Proceed as directed above.

Tolerances: NLT 75% (Q) of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

- **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227):** Meet the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label Tablets that must be chewed to indicate that they are to be chewed before swallowing.
- **USP REFERENCE STANDARDS (11)**  
USP Acetaminophen RS

## Acetaminophen Extended-Release Tablets

### DEFINITION

Acetaminophen Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ).

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

Sample: A portion of powdered Tablets

Acceptance criteria: Meet the requirements

- **B.** The retention time of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

## ASSAY

### PROCEDURE

Solution A: Phosphoric acid and water (1:9)

Mobile phase: Methanol, Solution A, and water (300:1:700)

Standard solution: 0.65 mg/mL of USP Acetaminophen RS in Mobile phase. Prepare by first dissolving in methanol, and then diluting with Mobile phase to volume.

Sample stock solution: Transfer 10 Tablets to a 250-mL volumetric flask containing 50 mL of water and a magnetic stir bar. Stir for at least 30 min or until the coating has dissolved. Add 150 mL of methanol, and stir for 45 min. Tablet cores should be disintegrated at least 15 min before ending the stirring. Remove the magnetic stir bar, and rinse into the flask with methanol. Dilute with methanol to volume, and centrifuge. Use the clear supernatant.

Sample solution: Dilute 5 mL of the Sample stock solution with Mobile phase to 200 mL.

### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 295 nm

Column: 3.9-mm  $\times$  15-cm; packing L1

Flow rate: 2.0 mL/min

Injection volume: 20  $\mu$ L

### System suitability

Sample: Standard solution

### Suitability requirements

Tailing factor: NMT 3.0

Relative standard deviation: NMT 2.0%

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution



- $C_s$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)  
 Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION** (711)**Test 1**

**Medium:** Simulated gastric fluid TS (without enzyme); 900 mL

**Apparatus 2:** 50 rpm

**Times:** 15 min, 1 h, and 3 h

**Standard solution:** A known concentration of USP Acetaminophen RS in *Medium*

**Sample solution:** A filtered portion of the solution under test, suitably diluted with *Medium* to obtain a concentration similar to that of the *Standard solution*

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** 280 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) dissolved.

**Tolerances:** See *Table 1*.

**Table 1**

Time	Amount Dissolved
15 min	45%–65%
1 h	60%–85%
3 h	NLT 85%

The percentages of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**For gelatin-coated Tablets**

**Medium, Apparatus, Standard solution, Sample solution, Instrumental conditions, and Analysis:** Proceed as directed in *Test 1*.

**Times:** 30 min, 90 min, and 4 h

**Tolerances:** See *Table 2*.

**Table 2**

Time	Amount Dissolved
30 min	40%–60%
90 min	55%–85%
4 h	NLT 80%

The percentages of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium, Apparatus, Standard solution, Sample solution, Instrumental conditions, and Analysis:** Proceed as directed in *Test 1*.

**Times:** 15 min, 1 h, and 3 h

**Tolerances:** See *Table 3*.

**Table 3**

Time	Amount Dissolved
15 min	40%–60%
1 h	55%–75%
3 h	NLT 80%

The percentages of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**IMPURITIES**

- **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** (227): Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where the Tablets are gelatin-coated, the label so states. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)  
 USP Acetaminophen RS

**Acetaminophen and Aspirin Tablets**

» Acetaminophen and Aspirin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Acetaminophen RS

USP Aspirin RS

USP Salicylic Acid RS

**Identification**—The relative retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution, Procedure for a Pooled Sample** (711)—

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

**Mobile phase**—Prepare as directed under *Assay*.

**Solvent mixture**—Prepare as directed under *Assay*.

**Internal standard solution**—Prepare a solution of benzoic acid in methanol having a concentration of about 1 mg per mL.

**Standard preparation I**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in the *Solvent mixture* to obtain a solution having a known concentration of about 70  $\mu$ g per mL. Combine 4.0 mL of this solution and 1.0 mL of the *Internal standard solution*, and mix.

**Standard preparation II**—Dissolve accurately weighed quantities of USP Acetaminophen RS and USP Aspirin RS in the *Solvent mixture* to obtain a solution having known concentrations of about 360  $\mu$ g of acetaminophen and about 360  $\mu$ g of aspirin per mL. Combine 4.0 mL of this solution and 1.0 mL of the *Internal standard solution*, and mix.

**Test preparation**—Combine 4.0 mL of a filtered portion of the solution under test and 1.0 mL of the *Internal standard solution*, and mix.

**Chromatographic system**—Proceed as directed under *Assay*.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the two *Standard preparations* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.3 for acetaminophen, 0.4 for salicylic acid, 0.6 for aspirin, and 1.0 for benzoic acid. Deter-



mine the amount of acetaminophen ( $C_8H_9NO_2$ ) dissolved by the formula:

$$90(C/W)(R_U / R_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Acetaminophen RS in *Standard preparation II*;  $R_U$  and  $R_S$  are the relative peak response ratios obtained from the *Test preparation* and *Standard preparation II*, respectively; and W is the labeled amount, in mg, of acetaminophen. Determine the amount of aspirin ( $C_9H_8O_4$ ) dissolved by the formula:

$$\{[90C_1(R_{U1} / R_{S1})] + [90C_2(R_{U2} / R_{S2})(1.3044)]\} / W$$

in which  $C_1$  and  $C_2$  are the concentrations, in  $\mu\text{g}$  per mL, of USP Aspirin RS in *Standard preparation II* and USP Salicylic Acid RS in *Standard preparation I*, respectively;  $R_{U1}$  and  $R_{S1}$  are the relative peak response ratios for the aspirin peak and the internal standard peak obtained from the *Test preparation* and *Standard preparation II*, respectively;  $R_{U2}$  and  $R_{S2}$  are the relative peak response ratios for the salicylic acid peak and the internal standard peak obtained from the *Test preparation* and *Standard preparation I*, respectively; and W is the labeled amount, in mg, of aspirin.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of  $C_8H_9NO_2$  and  $C_9H_8O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity* with respect to acetaminophen and to aspirin.

#### Limit of salicylic acid—

**Solvent mixture, Mobile phase, Internal standard solution, and Chromatographic system**—Prepare as directed in the Assay.

**Procedure**—Dissolve a suitable quantity of USP Salicylic Acid RS, accurately weighed, in *Solvent mixture* to obtain a solution having a known concentration of about 1.0 mg per mL. Transfer 1.0-mL, 5.0-mL, and 10.0-mL portions, respectively, of this solution to separate 100-mL volumetric flasks, add 10.0 mL of *Internal standard solution* to each flask, dilute with *Solvent mixture* to volume, and mix. Chromatograph these three Standard solutions as directed in the Assay. Plot the ratios of the peak responses for salicylic acid and benzoic acid for each of the Standard solutions versus concentrations, in mg per mL, of salicylic acid, and draw the straight line best fitting the three plotted points. From the graph so obtained, and from the ratio of the peak responses for salicylic acid and benzoic acid in the chromatogram of the *Assay preparation* as obtained in the Assay, determine the concentration, in mg per mL, of salicylic acid ( $C_7H_6O_3$ ) in the *Assay preparation*, and calculate the percentage of salicylic acid in relation to the concentration of aspirin in the *Assay preparation*, as determined in the Assay. Not more than 3.0% is found.

**Assay**—[NOTE—Use clean, dry glassware. Inject the *Standard preparation* and the *Assay preparation* promptly after preparation.]

**Solvent mixture**—Prepare a mixture of chloroform, methanol, and glacial acetic acid (78:20:2).

**Mobile phase**—Transfer 225 mg of tetramethylammonium hydroxide pentahydrate to a 1000-mL flask, and add 750 mL of water, 125 mL of methanol, 125 mL of acetonitrile, and 1.0 mL of glacial acetic acid. Stir for 3 minutes, pass through a membrane filter having a 0.5- $\mu\text{m}$  or finer porosity, and degas.

**Internal standard solution**—Dissolve benzoic acid in *Solvent mixture* to obtain a solution having a concentration of about 20 mg per mL.

**Standard preparation**—Transfer about 325 mg of USP Acetaminophen RS and about 325 mg of USP Aspirin RS, each accurately weighed, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of acetaminophen, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution* and about 50 mL of *Solvent mixture*, and sonicate for about 3 minutes. Dilute with *Solvent mixture* to volume, and mix. Pass a portion of this solution through a filter having a 2.5- $\mu\text{m}$  or finer porosity, and use the filtrate as the *Assay preparation*.

**Chromatographic system**—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph four replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for either analyte is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention times are about 2, 3, 5, and 8 minutes for acetaminophen, salicylic acid (if present), aspirin, and benzoic acid, respectively. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$100C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of acetaminophen and benzoic acid obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the same formula, except to read "USP Aspirin RS" where "USP Acetaminophen RS" is specified, and "aspirin" where "acetaminophen" is specified.

## Acetaminophen, Aspirin, and Caffeine Tablets

» Acetaminophen, Aspirin, and Caffeine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

#### USP Reference standards (11)—

USP Acetaminophen RS  
USP Aspirin RS  
USP Caffeine RS  
USP Salicylic Acid RS

**Identification**—The relative retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the Assay.

#### Dissolution (711)—

Medium: water; 900 mL.

Apparatus 2: 100 rpm.

Time: 60 minutes.

**Mobile phase, Internal standard solution, Solvent mixture, Standard stock solution, and Chromatographic system**—Proceed as directed in the Assay.

**Standard preparation**—Transfer 20.0 mL of *Standard stock solution*, 3.0 mL of *Internal standard solution*, and 20 mL of water to a 50-mL volumetric flask, mix, and allow to stand



for about 30 seconds. Dilute with *Solvent mixture* to volume, and mix. Use within 8 hours.

**Test preparation**—Transfer 20.0 mL of a filtered portion of the solution under test to a 50-mL volumetric flask, add 3.0 mL of *Internal standard solution* and 20 mL of *Solvent mixture*, mix, and allow to stand for 30 seconds. Dilute with *Solvent mixture* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay*. Calculate the quantities, in mg, of acetaminophen ( $C_8H_9NO_2$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) dissolved by the formula:

$$2250C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of the corresponding analyte and internal standard peaks of the solution under test and the *Standard preparation*, respectively.

**Tolerances**—Not less than 75% ( $Q$ ) of the labeled amounts of  $C_8H_9NO_2$ ,  $C_9H_8O_4$ , and  $C_8H_{10}N_4O_2$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity* with respect to acetaminophen, aspirin, and caffeine.

#### Limit of salicylic acid—

**Mobile phase and Solvent mixture**—Prepare as directed in the *Assay*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Solvent mixture* to obtain a solution having a known concentration of about 1 mg per mL. Transfer 2.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. This solution contains about 0.02 mg of USP Salicylic Acid RS per mL.

**Test preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of aspirin, to a 100-mL volumetric flask. Add about 75 mL of *Solvent mixture*, and shake by mechanical means for 30 minutes. Dilute with *Solvent mixture* to volume, and mix.

**Chromatographic system**—Proceed as directed for *Chromatographic system* in the *Assay*, except to use a 302-nm detector. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor is not more than 1.6; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the salicylic acid peaks. Calculate the percentage of salicylic acid in the portion of Tablets taken by the formula:

$$10,000(C/a)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*;  $a$  is the quantity, in mg, of aspirin in the portion of Tablets taken, based on the labeled amount; and  $r_U$  and  $r_S$  are the salicylic acid peak responses of the *Test preparation* and the *Standard preparation*, respectively: not more than 3.0% is found.

#### Assay—

**Mobile phase**—Prepare a suitable mixture of water, methanol, and glacial acetic acid (69:28:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of benzoic acid in methanol containing about 6 mg per mL.

**Solvent mixture**—Prepare a mixture of methanol and glacial acetic acid (95:5).

**Standard stock solution**—Dissolve accurately weighed quantities of USP Acetaminophen RS, USP Aspirin RS, and USP Caffeine RS in *Solvent mixture* to obtain a solution having known concentrations of about 0.25 mg of USP Acetaminophen RS per mL, 0.25/ $j$  mg of USP Aspirin RS per mL, and 0.25/ $j'$  mg of USP Caffeine RS per mL,  $j$  being the ratio of the labeled amount, in mg, of aspirin to the labeled amount, in mg, of acetaminophen per Tablet; and  $j'$  being the ratio of the labeled amount, in mg, of caffeine to the labeled amount, in mg, of acetaminophen per Tablet.

**Standard preparation**—Transfer 20.0 mL of *Standard stock solution* and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix. This solution contains about 0.1 mg of USP Acetaminophen RS, 0.1/ $j$  mg of USP Aspirin RS, and 0.1/ $j'$  mg of USP Caffeine RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of acetaminophen, to a 100-mL volumetric flask. Add about 75 mL of *Solvent mixture*, and shake by mechanical means for 30 minutes. Dilute with *Solvent mixture* to volume, and mix. Transfer 2.0 mL of this solution and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

**Chromatographic system**—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm  $\times$  10-cm column that contains 5- $\mu$ m packing L1, and is maintained at  $45 \pm 1^\circ$ . The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor for each analyte peak is not more than 1.2; the resolution,  $R$ , between any of the analyte and internal standard peaks is not less than 1.4; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.3 for acetaminophen, 0.5 for caffeine, 0.8 for aspirin, 1.0 for benzoic acid, and 1.2 for salicylic acid. Calculate the quantities, in mg, of acetaminophen ( $C_8H_9NO_2$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) in the portion of Tablets taken by the formula:

$$2500C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of the corresponding analyte and internal standard peaks of the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen and Caffeine Tablets

### DEFINITION

Acetaminophen and Caffeine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and caffeine ( $C_8H_{10}N_4O_2$ ).

### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, relative to the internal standard, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Solution A:** Methanol and glacial acetic acid (95:5)

**Mobile phase:** Methanol, glacial acetic acid, and water (28:3:69)



**Internal standard solution:** 6 mg/mL of benzoic acid in methanol

**Standard stock solution:** 0.25 mg/mL of USP Acetaminophen RS and 0.25/ mg/mL of USP Caffeine RS in *Solution A*; *J* being the ratio of the labeled amount, in mg, of caffeine to the labeled amount, in mg, of acetaminophen per Tablet

**Standard solution:** 0.1 mg/mL of USP Acetaminophen RS and 0.1/ mg/mL of USP Caffeine RS, prepared by transferring 20.0 mL of *Standard stock solution* and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and diluting with *Solution A* to volume

**Sample stock solution:** Nominally 2.5 mg/mL of acetaminophen in *Solution A*, prepared as follows. Transfer a portion of the powder equivalent to 250 mg of acetaminophen, from NLT 20 finely powdered Tablets, to a 100-mL volumetric flask. Add 75 mL of *Solution A*, and shake by mechanical means for 30 min. Dilute with *Solution A* to volume.

**Sample solution:** Transfer 2.0 mL of the *Sample stock solution* and 3.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Solution A* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 275 nm

**Column:** 4.6-mm × 10-cm; 5-μm packing L1

**Column temperature:** 45 ± 1°

**Flow rate:** 2 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for acetaminophen, caffeine, and benzoic acid are about 0.3, 0.5, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.4 between any of the analyte and internal standard peaks

**Tailing factor:** NMT 1.2 for each analyte peak

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate individually the percentages of the labeled amounts of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) and caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

*R<sub>U</sub>* = peak response ratio of acetaminophen or caffeine to the internal standard from the *Sample solution*

*R<sub>S</sub>* = peak response ratio of acetaminophen or caffeine to the internal standard from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Acetaminophen RS or USP Caffeine RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of acetaminophen or caffeine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) and caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>)

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 100 rpm

**Time:** 60 min

**Solution A, Mobile phase, Internal standard solution, Standard stock solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** Transfer 20.0 mL of the *Standard stock solution*, 3.0 mL of *Internal standard solution*, and 20 mL of water to a 50-mL volumetric flask, and allow

to stand for 30 s. Dilute with *Solution A* to volume. Use within 8 h.

**Sample solution:** Transfer an aliquot of a filtered portion of the solution under test to a 50-mL volumetric flask to obtain an expected concentration of 0.1 mg/mL of acetaminophen and 0.1/ mg/mL of caffeine, where *J* is defined for the *Standard stock solution*. Add 3.0 mL of *Internal standard solution* and 20 mL of *Solution A*, and allow to stand for 30 s. Dilute with *Solution A* to volume.

**Analysis:** Proceed as directed in the *Assay*, using the *Standard solution* and *Sample solution* prepared within the *Dissolution* test.

Calculate the percentages of the labeled amounts of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) and caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>) dissolved:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

*R<sub>U</sub>* = peak response ratio of acetaminophen or caffeine to the internal standard from the *Sample solution*

*R<sub>S</sub>* = peak response ratio of acetaminophen or caffeine to the internal standard from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Acetaminophen RS or USP Caffeine RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of acetaminophen or caffeine in the *Sample solution* (mg/mL)

**Tolerances:** NLT 75% (*Q*) of the labeled amounts of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>) and caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

- **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227):** Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Acetaminophen RS  
USP Caffeine RS

### Capsules Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

» Capsules Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>), chlorpheniramine maleate (C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>), dextromethorphan hydrobromide (C<sub>18</sub>H<sub>25</sub>NO · HBr · H<sub>2</sub>O), and pseudoephedrine hydrochloride (C<sub>10</sub>H<sub>15</sub>NO · HCl) or pseudoephedrine sulfate [(C<sub>10</sub>H<sub>15</sub>NO)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub>].  
NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The



name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Acetaminophen RS  
USP Chlorpheniramine Maleate RS  
USP Dextromethorphan Hydrobromide RS  
USP Pseudoephedrine Hydrochloride RS  
USP Pseudoephedrine Sulfate RS

**Labeling**—The label for each article encompassed by this monograph bears a name composed of the active ingredients contained in the article. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article.

**Identification**—

**A:** If pseudoephedrine hydrochloride or pseudoephedrine sulfate is purported to be present, the retention time of the major peak for pseudoephedrine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate*.

**B:** If acetaminophen is claimed in the labeling to be present, the retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetaminophen*.

**C:** If chlorpheniramine maleate is claimed in the labeling to be present, the retention time of the major peak for chlorpheniramine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.

**D:** If dextromethorphan hydrobromide is claimed in the labeling to be present, the retention time of the major peak for dextromethorphan in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for dextromethorphan hydrobromide*.

**Dissolution, Procedure for a Pooled Sample** (711)—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

**Test preparation**—Mix 9.0 mL of a filtered portion of the solution under test with 1.0 mL of 1% phosphoric acid solution.

**Procedure**—Determine the amounts of pseudoephedrine hydrochloride or pseudoephedrine sulfate (as appropriate), acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide dissolved, employing the procedures set forth in the *Assay for pseudoephedrine hydrochloride* or *Assay for pseudoephedrine sulfate*, *Assay for acetaminophen*, *Assay for chlorpheniramine maleate*, and *Assay for dextromethorphan hydrobromide*, respectively, making any necessary volumetric adjustments.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) or pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ], acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), and dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) are dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay for pseudoephedrine hydrochloride** (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

**Chlorpheniramine standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

**Dextromethorphan standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

**System suitability solution 1** (for Capsules that contain either all four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

**System suitability solution 2** (for Capsules that contain no chlorpheniramine)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

**Assay preparation**—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with water to obtain a solution having a concentration of about 0.12 mg of pseudoephedrine hydrochloride per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the Capsules taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; L is the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Capsule; D is the concentration, in mg per mL, of pseudoephedrine hydrochloride in the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Capsule and the extent of dilution; and  $r_U$  and  $r_S$  are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for pseudoephedrine sulfate** (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

**Mobile phase, System suitability solutions, and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

**Chlorpheniramine standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

**Dextromethorphan standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 2.5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.

**Assay preparation**—Proceed as directed for the *Assay preparation* in the *Assay for pseudoephedrine hydrochloride* to ob-



tain a solution having a concentration of about 0.24 mg of pseudoephedrine sulfate per mL.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for pseudoephedrine hydrochloride*. Calculate the quantity, in mg, of pseudoephedrine sulfate  $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$  in each Capsule taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

**Assay for acetaminophen (if present)**—

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (79:20:1). Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 25 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 4 mL of methanol, and mix until solution is complete. Add 0.2 mL of phosphoric acid, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.25 mg per mL.

**Assay preparation**—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.25 mg of acetaminophen per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the acetaminophen peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each Capsule taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; L is the labeled quantity, in mg, of acetaminophen in each Capsule; D is the concentration, in mg per mL, of acetaminophen in each mL of the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of acetaminophen in each Capsule, and the extent of dilution; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for chlorpheniramine maleate (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following*—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8  $\mu$ g per mL.

**Assay preparation**—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about

100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 8  $\mu$ g of chlorpheniramine maleate per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in each Capsule taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; L is the labeled quantity, in mg, of chlorpheniramine maleate in each Capsule; D is the concentration, in mg per mL, of chlorpheniramine maleate in each mL of the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of chlorpheniramine maleate in each Capsule, and the extent of dilution; and  $r_U$  and  $r_S$  are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextromethorphan hydrobromide (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following*—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.4 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 0.04 mg per mL.

**Assay preparation**—Transfer not fewer than 10 Capsules, accurately counted, to a 500-mL volumetric flask. Add about 100 mL of water and 10 mL of 5% phosphoric acid, and gently heat until the Capsules are fully dispersed. Cool the solution to room temperature, dilute with water to volume, mix, and filter. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.04 mg of dextromethorphan hydrobromide per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in each Capsule taken by the formula:

$$(370.33/352.32)(CL/D)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively; C is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; L is the labeled quantity, in mg, of dextromethorphan hydrobromide in each Capsule; D is the concentration, in mg per mL, of dextromethorphan hydrobromide in each mL of the *Assay preparation*, based on the number of Capsules taken, the labeled quantity, in mg, of dextromethorphan hydrobromide in each Capsule, and the extent of dilution; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Oral Powder Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

» Oral Powder Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ), and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) or pseudoephedrine sulfate ( $[C_{10}H_{15}NO]_2 \cdot H_2SO_4$ ).

NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Acetaminophen RS  
USP Chlorpheniramine Maleate RS  
USP Dextromethorphan Hydrobromide RS  
USP Pseudoephedrine Hydrochloride RS  
USP Pseudoephedrine Sulfate RS

**Labeling**—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article.

### Identification—

**A:** If pseudoephedrine hydrochloride or pseudoephedrine sulfate is claimed in the labeling to be present, the retention time of the major peak for pseudoephedrine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate*.

**B:** If acetaminophen is claimed in the labeling to be present, the retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetaminophen*.

**C:** If chlorpheniramine maleate is claimed in the labeling to be present, the retention time of the major peak for chlorpheniramine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.

**D:** If dextromethorphan hydrobromide is claimed in the labeling to be present, the retention time of the major peak for dextromethorphan in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for dextromethorphan hydrobromide*.

**Minimum fill (755):** meets the requirements.

### Uniformity of dosage units (905)—

FOR ORAL POWDER PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Assay for pseudoephedrine hydrochloride** (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

*Chlorpheniramine standard preparation*—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

*Dextromethorphan standard preparation*—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, dilute with 0.1% phosphoric acid to volume, and mix.

*System suitability solution 1* (for Oral Powder that contains either all four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

*System suitability solution 2* (for Oral Powder that contains no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

*Assay preparation*—Transfer the contents of 10 unit-dose containers of the Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2.0 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.24 mg of pseudoephedrine hydrochloride per mL.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ), in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; L is the labeled quantity, in mg, of pseudoephedrine hydrochloride in each unit-dose container; D is the concentration, in mg per mL, of pseudoephedrine hydrochloride in each mL of the *Assay preparation*, based on the number of unit-dose containers taken, the labeled quantity, in mg, of pseudoephedrine hydrochloride in each unit-dose container, and the extent of dilution; and  $r_U$  and  $r_S$  are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for pseudoephedrine sulfate** (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride* under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.



**Chlorpheniramine standard preparation**—Prepare as directed for *Standard preparation* in the Assay for chlorpheniramine maleate.

**Dextromethorphan standard preparation**—Prepare as directed for *Standard preparation* in the Assay for dextromethorphan hydrobromide.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 6.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, dilute with 0.1% phosphoric acid to volume, and mix.

**System suitability solution 1** (for Oral Powder that contains either all four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

**System suitability solution 2** (for Oral Powder that contains no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

**Assay preparation**—Proceed as directed for the Assay preparation in the Assay for pseudoephedrine hydrochloride to obtain a solution having a concentration of about 0.48 mg of pseudoephedrine sulfate per mL.

**Procedure**—Proceed as directed for *Procedure* in the Assay for pseudoephedrine hydrochloride. Calculate the quantity, in mg, of pseudoephedrine sulfate  $[(C_{10}H_{15}NO)_2 \cdot H_2SO_4]$  in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

#### Assay for acetaminophen (if present)—

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the Assay for pseudoephedrine hydrochloride under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

**Assay preparation**—Transfer the contents of 10 unit-dose containers of the Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of about 0.50 mg of acetaminophen per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; L is the labeled quantity, in mg, of acetaminophen in each unit-dose container; D is the concentration, in mg per mL, of acetaminophen in the *Assay preparation*, based on the number of unit-dose containers taken, the labeled quantity, in mg, of acetaminophen in each unit-dose container, and the extent of dilution; and  $r_U$  and  $r_S$  are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for chlorpheniramine maleate (if present)—

**Mobile phase and Chromatographic system**—Proceed as directed in the Assay for pseudoephedrine hydrochloride under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8  $\mu$ g per mL.

**Assay preparation**—Transfer the contents of 10 unit-dose containers of Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. Quantitatively dilute a portion of this solution, if necessary, with 0.1% phosphoric acid to obtain a solution having a concentration of 8  $\mu$ g of chlorpheniramine maleate per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in each unit-dose container of Oral Powder taken by the formula:

$$(CL/D)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; L is the labeled quantity, in mg, of chlorpheniramine maleate in each unit-dose container; D is the concentration, in mg per mL, of chlorpheniramine maleate in each mL of the *Assay preparation*, based on the number of unit-dose containers taken, the labeled quantity, in mg, of chlorpheniramine maleate in each unit-dose container, and the extent of dilution; and  $r_U$  and  $r_S$  are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for dextromethorphan hydrobromide (if present)—

**Mobile phase and Chromatographic system**—Proceed as directed in the Assay for pseudoephedrine hydrochloride under *Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of 0.08 mg per mL.

**Assay preparation**—Transfer the contents of 10 unit-dose containers of Oral Powder to a 2000-mL volumetric flask. Add 1000 mL of water and 2 mL of phosphoric acid. Gently heat to about 60° until the powder is fully dispersed. Cool the flask to room temperature, add 40 mL of methanol, dilute with water to volume, and mix. If necessary, quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a concentration of 0.08 mg of dextromethorphan hydrobromide per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan



hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in each unit-dose container of Oral Powder taken by the formula:

$$(370.33/352.32)(CL/D)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively;  $C$  is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*;  $L$  is the labeled quantity, in mg, of dextromethorphan hydrobromide in each unit-dose container;  $D$  is the concentration, in mg per mL, of dextromethorphan hydrobromide in each mL of the *Assay preparation*, based on the number of unit-dose containers taken, the labeled quantity, in mg, of dextromethorphan hydrobromide in each unit-dose container, and the extent of dilution; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Oral Solution Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

» Oral Solution Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ), and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) or pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ].

NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

#### USP Reference standards (11)—

USP Acetaminophen RS  
USP Chlorpheniramine Maleate RS  
USP Dextromethorphan Hydrobromide RS  
USP Pseudoephedrine Hydrochloride RS  
USP Pseudoephedrine Sulfate RS

**Labeling**—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article.

#### Identification—

A: If pseudoephedrine hydrochloride or pseudoephedrine sulfate is claimed in the labeling to be present, the retention time of the major peak for pseudoephedrine in the chromat-

ogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate*.

B: If acetaminophen is claimed in the labeling to be present, the retention time of the major peak for acetaminophen in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for acetaminophen*.

C: If chlorpheniramine maleate is claimed in the labeling to be present, the retention time of the major peak for chlorpheniramine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.

D: If dextromethorphan hydrobromide is claimed in the labeling to be present, the retention time of the major peak for dextromethorphan in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for dextromethorphan hydrobromide*.

#### Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

#### Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 3.7 and 7.5.

**Alcohol Determination, Method II** (611) (if present): between 90.0% and 110.0% of the labeled amount of  $C_2H_5OH$ .

**Microbial enumeration tests (61) and Absence of specified microorganisms (62)**—The total bacterial count does not exceed 100 cfu per g, the total combined molds and yeasts count does not exceed 10 cfu per g, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

**Assay for pseudoephedrine hydrochloride** (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.15 g of triethylamine hydrochloride, 0.25 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 1.5 mg per mL. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, add 8.0 mL of *Mobile phase*, dilute with water to volume, and mix.

**Chlorpheniramine standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

**Dextromethorphan standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

**System suitability solution 1** (for Oral Solution that contains either all the four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

**System suitability solution 2** (for Oral Solution that contains no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

**Assay preparation**—Transfer an accurately measured volume of the Oral Solution, equivalent to 15 mg of pseudoephedrine hydrochloride, to a 100-mL volumetric flask, add



80.0 mL of *Mobile phase*, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the pseudoephedrine peak is not greater than 2.5; and the relative standard deviation for replicate injections is not more than 2.0%. Separately inject about 10  $\mu$ L of *System suitability solution 1* or *System suitability solution 2*, as appropriate. The resolution, *R*, between pseudoephedrine and chlorpheniramine or between pseudoephedrine and dextromethorphan is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; *V* is the volume, in mL, of the Oral Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for pseudoephedrine sulfate** (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

**Mobile phase, System suitability solutions, and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*.

**Chlorpheniramine standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for chlorpheniramine maleate*.

**Dextromethorphan standard preparation**—Prepare as directed for *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, add 4.0 mL of *Mobile phase*, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to 30 mg of pseudoephedrine sulfate, to a 100-mL volumetric flask, add 80.0 mL of *Mobile phase*, dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for pseudoephedrine hydrochloride*. Calculate the quantity, in mg, of pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ] in each mL of the Oral Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

**Assay for acetaminophen (if present)**—

**Mobile phase**—Prepare a suitable degassed and filtered mixture of water, methanol, and glacial acetic acid (79:20:1). Make any necessary adjustments (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 16.5 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 2.5 mL of methanol, and mix until solution is complete. Dilute with water to volume, and mix to obtain

a solution having a known concentration of about 0.165 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 33 mg of acetaminophen, to a 200-mL volumetric flask, add 5 mL of methanol, and mix. Dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the acetaminophen peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each mL of the Oral Solution taken by the formula:

$$200(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; *V* is the volume, in mL, of the Oral Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for chlorpheniramine maleate (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 1 mg per mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, add 80 mL of *Mobile phase*, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 1 mg of chlorpheniramine maleate, to a 100-mL volumetric flask. Add 80 mL of *Mobile phase*, dilute with water to volume, and mix.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the Oral Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; *V* is the volume, in mL, of the Oral Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextromethorphan hydrobromide (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 1.5 mg per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 80 mL of *Mobile phase*, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 7.5 mg of dextromethorphan hydrobromide, to a 100-mL volumetric flask,



add 80 mL of *Mobile phase*, dilute with water to volume, and mix.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in each mL of the Oral Solution taken by the formula:

$$(370.33/352.32)(100C/V)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively; C is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; V is the volume, in mL, of the Oral Solution taken; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine

» Tablets Containing at Least Three of the Following—Acetaminophen and Salts of Chlorpheniramine, Dextromethorphan, and Pseudoephedrine contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ), and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) or pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ]. NOTE—The heading of this monograph does not constitute the official title. It is not intended that the name described herein be recognized as the official title or the common or usual name. The name for each article encompassed by this monograph shall be composed of the names of the active ingredients contained therein, as well as the quantitative amount of each active ingredient, and a statement of the function (or purpose) of the ingredient in the article.

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

#### USP Reference standards (11)—

USP Acetaminophen RS  
USP Chlorpheniramine Maleate RS  
USP Dextromethorphan Hydrobromide RS  
USP Pseudoephedrine Hydrochloride RS  
USP Pseudoephedrine Sulfate RS

**Labeling**—The label for each article encompassed by this monograph bears a name composed of the active ingredients. The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

#### Identification—

A: If pseudoephedrine hydrochloride or pseudoephedrine sulfate is claimed in the labeling to be present, the chromat-

ogram of the *Assay preparation*, obtained as directed in the *Assay for pseudoephedrine hydrochloride* or the *Assay for pseudoephedrine sulfate*, exhibits a major peak for pseudoephedrine, the retention time of which corresponds to that exhibited by the *Standard preparation*.

B: If acetaminophen is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for acetaminophen*, exhibits a major peak for acetaminophen, the retention time of which corresponds to that exhibited by the *Standard preparation*.

C: If chlorpheniramine maleate is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for chlorpheniramine maleate*, exhibits a major peak for chlorpheniramine, the retention time of which corresponds to that exhibited by the *Standard preparation*.

D: If dextromethorphan hydrobromide is claimed in the labeling to be present, the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for dextromethorphan hydrobromide*, exhibits a major peak for dextromethorphan, the retention time of which corresponds to that exhibited by the *Standard preparation*.

#### Dissolution, Procedure for a Pooled Sample (711)—

##### TEST 1—

**Medium:** pH 5.8 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

**Test preparation**—Mix 9.0 mL of a filtered portion of the solution under test with 1.0 mL of 1% phosphoric acid solution.

**Procedure**—Determine the amounts of pseudoephedrine hydrochloride or pseudoephedrine sulfate (as appropriate), acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide dissolved, employing the procedures set forth in the *Assay for pseudoephedrine hydrochloride* or *Assay for pseudoephedrine sulfate*, *Assay for acetaminophen*, *Assay for chlorpheniramine maleate*, and *Assay for dextromethorphan hydrobromide*, respectively, making any necessary volumetric adjustments.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) or pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ], acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), and dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) are dissolved in 45 minutes.

**TEST 2**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** water; 900 mL.

**Apparatus, Time, Test preparation, Procedure, and Tolerances**—Proceed as directed for *Test 1*.

**Uniformity of dosage units (905):** meet the requirements.

**Assay for pseudoephedrine hydrochloride** (where pseudoephedrine hydrochloride is the salt form used, if present in the formulation)—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.3 g of triethylamine hydrochloride, 0.15 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 1.0 mL of this solution to a 25-mL volumetric flask, add 2.5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.



**Chlorpheniramine standard preparation**—Prepare as directed for the *Standard preparation* in the *Assay for chlorpheniramine maleate*.

**Dextromethorphan standard preparation**—Prepare as directed for the *Standard preparation* in the *Assay for dextromethorphan hydrobromide*.

**System suitability solution 1** (for Tablets that contain either all the four ingredients or a combination of three containing chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Chlorpheniramine standard preparation*.

**System suitability solution 2** (for Tablets that contain no chlorpheniramine salt)—Mix equal volumes of the *Standard preparation* and the *Dextromethorphan standard preparation*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 6 mg of pseudoephedrine hydrochloride, to a 50-mL volumetric flask. Add 5 mL of methanol, and sonicate to disperse the powder. Dilute with 0.1% phosphoric acid to volume, mix, and filter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the pseudoephedrine peak is not greater than 2.5, and the relative standard deviation for replicate injections is not more than 2.0%. Separately inject about 10 µL of *System suitability solution 1* or *System suitability solution 2*, as appropriate. The resolution, *R*, between pseudoephedrine and chlorpheniramine or between pseudoephedrine and dextromethorphan is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$50C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*, and *r<sub>u</sub>* and *r<sub>s</sub>* are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for pseudoephedrine sulfate** (where pseudoephedrine sulfate is the salt form used, if present in the formulation)—

**Mobile phase, Chlorpheniramine standard preparation, Dextromethorphan standard preparation, System suitability solutions, and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Sulfate RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 2.5 mL of methanol, dilute with 0.1% phosphoric acid to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 12 mg of pseudoephedrine sulfate, to a 50-mL volumetric flask. Add 5 mL of methanol, and sonicate to disperse the powder. Dilute with 0.1% phosphoric acid to volume, mix, and filter.

**Procedure**—Proceed as directed for *Procedure* in the *Assay for pseudoephedrine hydrochloride*. Calculate the quantity, in

mg, of pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ] in the portion of Tablets taken by the formula:

$$50C(r_u / r_s)$$

in which the terms are as defined therein, pseudoephedrine sulfate being substituted for pseudoephedrine hydrochloride.

**Assay for acetaminophen (if present)**—

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and glacial acetic acid (79:20:1). Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 50 mg of USP Acetaminophen RS, accurately weighed, to a 100-mL volumetric flask. Add 4 mL of methanol, and mix until solution is complete. Dilute with 0.1% phosphoric acid to volume, and mix.

**Assay preparation**—Weigh and powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of acetaminophen, to a 50-mL volumetric flask. Add about 7.5 mL of methanol, and sonicate to disperse the powder. Add 0.5 mL of phosphoric acid, dilute with water to volume, mix, and filter. Transfer 25.0 mL of the filtered solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the acetaminophen peak is not greater than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the acetaminophen peaks. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$200C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the acetaminophen peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for chlorpheniramine maleate (if present)**—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 8 µg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2 mg of chlorpheniramine maleate, to a 250-mL volumetric flask. Add 25 mL of methanol, and sonicate to disperse the powder. Add 1 mL of phosphoric acid, dilute with water to volume, mix, and filter.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate



( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Tablets taken by the formula:

$$250C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses for chlorpheniramine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextromethorphan hydrobromide (if present)**—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay for pseudoephedrine hydrochloride*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Dextromethorphan Hydrobromide RS in water to obtain a solution having a known concentration of about 0.6 mg per mL. Quantitatively dilute a portion of this solution with 0.1% phosphoric acid to obtain a solution having a known concentration of about 0.06 mg per mL.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 6 mg of dextromethorphan hydrobromide, to a 100-mL volumetric flask. Add 10 mL of methanol, and sonicate to disperse the powder. Add 0.4 mL of phosphoric acid, dilute with water to volume, mix, and filter.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the dextromethorphan peaks. Calculate the quantity, in mg, of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in the portion of Tablets taken by the formula:

$$(370.33 / 352.32)(100C)(r_U / r_S)$$

in which 370.33 and 352.32 are the molecular weights of dextromethorphan hydrobromide monohydrate and anhydrous dextromethorphan hydrobromide, respectively;  $C$  is the concentration, in mg per mL, of USP Dextromethorphan Hydrobromide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the dextromethorphan peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acetaminophen, Chlorpheniramine Maleate, and Dextromethorphan Hydrobromide Tablets

### DEFINITION

Acetaminophen, Chlorpheniramine Maleate, and Dextromethorphan Hydrobromide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ), chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), and dextromethorphan hydrobromide monohydrate ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak for acetaminophen of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Acetaminophen*.
- **B.** The retention time of the major peak for chlorpheniramine of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Chlorpheniramine Maleate*.
- **C.** The retention time of the major peak for dextromethorphan of the *Sample solution* corresponds to that of the

*Standard solution*, as obtained in the *Assay for Dextromethorphan Hydrobromide*.

### ASSAY

#### Change to read:

#### • ACETAMINOPHEN

**Mobile phase:** Methanol, glacial acetic acid, and water (20:1:79)

**Standard solution:** 0.5 mg/mL of USP Acetaminophen RS prepared as follows. Transfer an appropriate amount of USP Acetaminophen RS to a suitable volumetric flask and add methanol using 4% of the final volume. Mix until solution is complete and dilute with 0.1% phosphoric acid to volume.

**Sample stock solution:** Nominally 2 mg/mL of acetaminophen prepared as follows. Transfer a portion of powdered Tablets (NLT 20), equivalent to 100 mg of acetaminophen, to a 50-mL volumetric flask. Add 7.5 mL of methanol, and sonicate to disperse the powder. Add 0.5 mL of phosphoric acid, dilute with water to volume, and filter.

**Sample solution:** Nominally 0.5 mg/mL of acetaminophen from *Sample stock solution* in water

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  15-cm;  $\Delta$ 5- $\mu$ m  $\Delta$ USP40 packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response of acetaminophen from the *Sample solution*

$r_S$  = peak response of acetaminophen from the *Standard solution*

$C_S$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ )

#### Change to read:

#### • CHLORPHENIRAMINE MALEATE

**Mobile phase:** Methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.3 g of triethylamine hydrochloride, 0.15 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution

$\Delta$ USP40

**Standard stock solution:** 0.8 mg/mL of USP Chlorpheniramine Maleate RS in water

**Standard solution:** 8  $\mu$ g/mL of USP Chlorpheniramine Maleate RS in 0.1% phosphoric acid from *Standard stock solution*

$\Delta$ USP40

**Sample solution:** Nominally 8  $\mu$ g/mL of chlorpheniramine maleate prepared as follows. Transfer a portion of powdered Tablets (NLT 20), equivalent to 2 mg of chlorpheniramine maleate, to a 250-mL volumetric



flask. Add 25 mL of methanol, and sonicate to disperse the powder. Add 1 mL of phosphoric acid, dilute with water to volume, and filter.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; 5-μm<sup>▲</sup>USP40 packing L11

Flow rate: 2 mL/min

Injection volume: 10 μL

#### System suitability

Samples: Standard solution<sup>▲</sup>USP40

#### Suitability requirements

<sup>▲</sup>USP40

Tailing factor: NMT 2.5<sup>▲</sup>USP40

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of chlorpheniramine maleate (C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of chlorpheniramine from the Sample solution

$r_s$  = peak response of chlorpheniramine from the Standard solution

$C_s$  = concentration of USP Chlorpheniramine Maleate RS in the Standard solution (mg/mL)

$C_u$  = nominal concentration of chlorpheniramine maleate in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of chlorpheniramine maleate (C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>)

#### Change to read:

#### • DEXTROMETHORPHAN HYDROBROMIDE

Mobile phase, <sup>▲</sup>USP40 Chromatographic system, and System suitability: Proceed as directed in the Assay for Chlorpheniramine Maleate.

Standard stock solution: 0.6 mg/mL of USP Dextromethorphan Hydrobromide RS in water

Standard solution: 0.06 mg/mL of USP Dextromethorphan Hydrobromide RS in 0.1% phosphoric acid, from Standard stock solution

<sup>▲</sup>USP40

Sample solution: Nominally 0.06 mg/mL of dextromethorphan hydrobromide prepared as follows. Transfer a portion of powdered Tablets (NLT 20), equivalent to 6 mg of dextromethorphan hydrobromide, to a 100-mL volumetric flask. Add 10 mL of methanol, and sonicate to disperse the powder. Add 0.4 mL of phosphoric acid, dilute with water to volume, and filter.

#### System suitability

Samples: Standard solution<sup>▲</sup>USP40

#### Suitability requirements

<sup>▲</sup>USP40

Tailing factor: NMT 2.5<sup>▲</sup>USP40

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of dextromethorphan hydrobromide monohydrate (C<sub>18</sub>H<sub>25</sub>NO · HBr · H<sub>2</sub>O) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

$r_u$  = peak response of dextromethorphan from the Sample solution

$r_s$  = peak response of dextromethorphan from the Standard solution

$C_s$  = concentration of USP Dextromethorphan Hydrobromide RS in the Standard solution (mg/mL)

$C_u$  = nominal concentration of dextromethorphan hydrobromide in the Sample solution (mg/mL)

$M_{r1}$  = molecular weight of dextromethorphan hydrobromide monohydrate, 370.32<sup>▲</sup>USP40

$M_{r2}$  = molecular weight of anhydrous dextromethorphan hydrobromide, 352.32

Acceptance criteria: 90.0%–110.0% of the labeled amount of dextromethorphan hydrobromide monohydrate (C<sub>18</sub>H<sub>25</sub>NO · HBr · H<sub>2</sub>O)

#### PERFORMANCE TESTS

- **DISSOLUTION (711), Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms, Procedure for a pooled sample for immediate-release dosage forms**

##### Test 1

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Sample solution: Mix 9.0 mL of a filtered portion of the solution with 1.0 mL of 1% phosphoric acid solution.

Analysis: Determine the percentage of the labeled amount of acetaminophen, chlorpheniramine maleate, and dextromethorphan hydrobromide dissolved, using the Analysis set forth in the Assay for Acetaminophen, the Assay for Chlorpheniramine Maleate, and the Assay for Dextromethorphan Hydrobromide, respectively, making any necessary volumetric adjustments.

Tolerances: NLT 75% (Q) of the labeled amount of acetaminophen (C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub>), chlorpheniramine maleate (C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>), and dextromethorphan hydrobromide monohydrate (C<sub>18</sub>H<sub>25</sub>NO · HBr · H<sub>2</sub>O) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

Medium: 0.1 M hydrochloric acid; 900 mL

Apparatus 2, Time, Sample solution, Analysis, and

Tolerances: Proceed as directed in Test 1.

Test 3: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 3.

Medium: pH 5.8 phosphate buffer (see Reagents, Indicators, and Solutions—Buffer Solutions); 900 mL

Apparatus 2, Time, Sample solution, Analysis, and

Tolerances: Proceed as directed in Test 1.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### Add the following:

- **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227):** Meet the requirements<sup>▲</sup>USP40

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** The label states the name and quantity of each active ingredient and indicates its function (or purpose) in the article. When more than one Dissolution Test is given, the labeling states the Dissolution Test used only if Test 1 is not used.



**Change to read:**

- **USP REFERENCE STANDARDS (11)**  
USP Acetaminophen RS  
USP Chlorpheniramine Maleate RS  
USP Dextromethorphan Hydrobromide RS

▲ USP40

**Acetaminophen and Codeine Phosphate Capsules**

» Acetaminophen and Codeine Phosphate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**USP Reference standards (11)**—

USP Acetaminophen RS  
USP Codeine Phosphate RS

**Identification**—

A: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Transfer a portion of Capsule contents, equivalent to about 12 mg of codeine phosphate, to a separator, add 5 mL of water, 1 mL of ammonium hydroxide, and 5 mL of methylene chloride, shake for 1 minute, and allow the layers to separate. Use the clear, lower layer as the test solution. Prepare a Standard solution of USP Acetaminophen RS and USP Codeine Phosphate RS in methanol containing 12 mg of each per mL. Apply 10  $\mu$ L of each solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of methanol and ammonium hydroxide (49:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wave-length UV light: the  $R_f$  values of the two principal spots obtained from the test solution correspond to those obtained from the Standard solution.

**Dissolution, Procedure for a Pooled Sample (711)**—

Medium: 0.01 N hydrochloric acid; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

**Procedure**—Determine the amounts of acetaminophen ( $C_8H_9NO_2$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) dissolved by employing the procedure set forth in the *Assay*, except to use 0.01 N hydrochloric acid to prepare the *Codeine phosphate standard stock solution* and to make any other necessary volumetric adjustments.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of  $C_8H_9NO_2$  and  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$  is dissolved in 30 minutes.

**Uniformity of dosage units (905):** meet the requirements.

**PROCEDURE FOR CONTENT UNIFORMITY**—

*Buffer solution, Mobile phase, Codeine phosphate standard stock solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay*.

**Sample preparation**—Transfer the contents of 1 Capsule to a 100-mL volumetric flask, add about 75 mL of *Mobile phase*, and sonicate for 10 minutes. Dilute with *Mobile phase* to volume, and mix. Transfer 5.0-mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable 1- $\mu$ m filter.

**Procedure**—Separately inject equal volumes (about 30  $\mu$ L) of the *Standard preparation* and the *Sample preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the Capsule taken by the formula:

$$1000C_A(r_U / r_S)$$

in which  $C_A$  is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Sample preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the Capsule taken by the formula:

$$(406.37/397.37)1000C_U(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $C_U$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; and the other terms are as defined herein.

**Assay**—

*Buffer solution, Mobile phase, Codeine phosphate standard stock solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay under Acetaminophen and Codeine Phosphate Tablets*.

**Assay preparation**—Remove as completely as possible the contents of not fewer than 20 Capsules, weigh, and mix. Transfer an accurately weighed portion of the combined contents, equivalent to about 300 mg of acetaminophen, to a 100-mL volumetric flask, add about 75 mL of *Mobile phase*, and sonicate for 10 minutes. Dilute with *Mobile phase* to volume, and mix. Transfer 5.0-mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable 1- $\mu$ m filter.

**Procedure**—Separately inject equal volumes (about 30  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Capsules taken by the formula:

$$(LC_A / C_U)(r_U / r_S)$$

in which  $L$  is the labeled quantity, in mg, of acetaminophen in each Capsule;  $C_A$  is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of acetaminophen in the *Assay preparation*, based upon the labeled quantity per Capsule and the extent of dilution; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Capsules taken by the formula:

$$(406.37/397.37)(LC_C / C_U)(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $L$  is the labeled quantity, in mg, of codeine phosphate hemihydrate in each Capsule;  $C_C$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*;  $C_U$  is the concentration, in mg



per mL, of codeine phosphate hemihydrate in the Assay preparation, based upon the labeled quantity per Capsule and the extent of dilution; and the other terms are as defined herein.

## Acetaminophen and Codeine Phosphate Oral Solution

### DEFINITION

Acetaminophen and Codeine Phosphate Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

### IDENTIFICATION

- A. The retention times of the major peaks of the *Sample solutions* correspond to those of the *Standard solutions*, as obtained in the Assays for Acetaminophen and Codeine Phosphate.

- B. **THIN-LAYER CHROMATOGRAPHY**

**Standard solution:** 12 mg/mL each of USP Acetaminophen RS and USP Codeine Phosphate RS in methanol

**Sample solution:** Transfer a volume of Oral Solution, equivalent to 12 mg of codeine phosphate, to a separator. Add 1 mL of ammonium hydroxide and 5 mL of methylene chloride. Shake for 1 min, and allow the layers to separate. Use the clear lower layer.

**Developing solvent system:** Methanol and ammonium hydroxide (49:1)

**Chromatographic system**

(See Chromatography (621), Thin-Layer Chromatography.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10  $\mu$ L

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Locate the spots on the plate by examination under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  values of the two principal spots of the *Sample solution* correspond to those of the *Standard solution*.

### ASSAY

#### Change to read:

- A. **ACETAMINOPHEN**

**Mobile phase:** Methanol and water (3:7)

**Standard solution:** 0.48 mg/mL of USP Acetaminophen RS in *Mobile phase*

**Sample solution:** Nominally 0.48 mg/mL of acetaminophen in *Mobile phase*, prepared by adding a volume of Oral Solution, equivalent to 120 mg of acetaminophen, to a 250-mL volumetric flask. Dilute with *Mobile phase* to volume.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

$\Delta$ USP40

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of acetaminophen from the *Sample solution*

$r_S$  = peak response of acetaminophen from the *Standard solution*

$C_S$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### Change to read:

- B. **CODEINE PHOSPHATE**

**Mobile phase:** Dissolve 4.44 g of docusate sodium in 1000 mL of a mixture of methanol, tetrahydrofuran, phosphoric acid, and water (600:40:1:360) with stirring, and pass through a membrane filter of 0.45- $\mu$ m or finer pore size.

**Diluent:** Methanol and water (3:7)

**Standard solution:** 0.12 mg/mL of USP Codeine Phosphate RS in *Diluent*

**Sample solution:** Nominally 0.12 mg/mL of codeine phosphate hemihydrate in *Diluent*, prepared by adding a volume of Oral Solution, equivalent to 12 mg of codeine phosphate hemihydrate, to a 100-mL volumetric flask. Dilute with *Diluent* to volume.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

$\Delta$ USP40

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of codeine phosphate from the *Sample solution*

$r_S$  = peak response of codeine phosphate from the *Standard solution*

$C_S$  = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of codeine phosphate in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of codeine phosphate hemihydrate, 406.37

$M_{r2}$  = molecular weight of anhydrous codeine phosphate, 397.37



Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905)  
For single-unit containers  
Acceptance criteria: Meets the requirements
- **DELIVERABLE VOLUME** (698)  
For multiple-unit containers  
Acceptance criteria: Meets the requirements

## IMPURITIES

### Add the following:

- ▲ **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** (227): Meets the requirements  $\Delta_{USP40}$

## SPECIFIC TESTS

- **PH** (791): 4.0–6.1
- **ALCOHOL DETERMINATION** (611), *Method II* (if present): 90.0%–120.0% of the labeled quantity of alcohol ( $C_2H_5OH$ ), acetone being used as the internal standard

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Acetaminophen RS  
USP Codeine Phosphate RS

# Acetaminophen and Codeine Phosphate Oral Suspension

## DEFINITION

Acetaminophen and Codeine Phosphate Oral Suspension is a suspension of Acetaminophen and Codeine Phosphate in a suitable aqueous vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

## IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHY**  
**Standard solution:** 12 mg/mL each of USP Acetaminophen RS and USP Codeine Phosphate RS in methanol  
**Sample solution:** Transfer a volume of Oral Suspension, equivalent to 12 mg of codeine phosphate, to a separator. Add 1 mL of ammonium hydroxide and 5 mL of methylene chloride, shake for 1 min, and allow the layers to separate. Use the clear lower layer.

## Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Developing solvent system:** Methanol and ammonium hydroxide (49:1)

**Application volume:** 10  $\mu$ L

## Analysis

**Samples:** *Standard solution* and *Sample solution*  
Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Locate the spots on the plate by examination under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  values of the two principal spots of the *Sample solution* correspond to those of the *Standard solution*.

## ASSAY

### Change to read:

## PROCEDURE

**Diluent:** Methanol and 0.01 N sodium hydroxide (30:70)

**Mobile phase:** Dissolve 4.9 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 3.9, and add 216 mg of sodium 1-octanesulfonate. Add 100 mL of acetonitrile, and filter.

**Codeine phosphate standard stock solution:** 0.5 mg/mL of USP Codeine Phosphate RS in *Diluent*

**Standard stock solution:** Transfer a quantity of 5J mg of USP Acetaminophen RS (J being the ratio of the labeled amount, in mg, of acetaminophen to the labeled amount, in mg, of codeine phosphate hemihydrate) and 10.0 mL of *Codeine phosphate standard stock solution* to a 100-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume.

**System suitability stock solution:** 0.02 mg/mL of sodium benzoate and 0.03 mg/mL of methylparaben in *Diluent*

**System suitability solution:** Transfer 10.0 mL of the *System suitability stock solution* to a 50-mL volumetric flask, add 10.0 mL of *Standard stock solution*, and dilute with *Mobile phase* to volume.

**Standard solution:** 0.01 mg/mL of USP Codeine Phosphate RS and 0.01J mg/mL of USP Acetaminophen RS in *Mobile phase*. Prepare by diluting 10.0 mL of the *Standard stock solution* with *Mobile phase* to 50 mL in a volumetric flask.

**Sample stock solution:** Nominally 0.5 mg/mL of acetaminophen  $\Delta$  and 0.5 mg/mL of codeine phosphate hemihydrate  $\Delta_{USP40}$  in *Diluent* prepared as follows. Transfer a measured volume of well-mixed Oral Suspension, equivalent to 50 mg of acetaminophen, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and mix by mechanical means for 30 min. Dilute with *Diluent* to volume. Foaming may be minimized by adding a few drops of acetonitrile before diluting with *Diluent* to volume. Centrifuge a portion of this mixture.

**Sample solution:** Dilute 10.0 mL of the clear supernatant from the *Sample stock solution* with *Mobile phase* to 50 mL in a volumetric flask.

## Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L11

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

## System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for acetaminophen, benzoate, codeine, and methylparaben are about 0.25, 0.5, 1.0, and 1.3, respectively.]

## Suitability requirements

**Resolution:** NLT 2 between each pair of adjacent peaks, *System suitability solution*

**Tailing factor:** NMT 2 for each analyte peak, *Standard solution*

$\Delta_{USP40}$

**Relative standard deviation:** NMT 2.0%, *Standard solution*

## Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response of acetaminophen from the *Sample solution*  
 $r_S$  = peak response of acetaminophen from the *Standard solution*  
 $C_S$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

Calculate the percentage of the labeled amount of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $r_U$  = peak response of codeine from the *Sample solution*  
 $r_S$  = peak response of codeine from the *Standard solution*  
 $C_S$  = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of codeine phosphate hemihydrate in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of codeine phosphate hemihydrate, 406.37  
 $M_{r2}$  = molecular weight of anhydrous codeine phosphate, 397.37

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

##### • UNIFORMITY OF DOSAGE UNITS (905)

For single-unit containers

Acceptance criteria: Meets the requirements

##### • DELIVERABLE VOLUME (698)

For multiple-unit containers

Acceptance criteria: Meets the requirements

#### IMPURITIES

Add the following:

##### ▲ 4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227): Meets the requirements▲<sup>USP40</sup>

#### SPECIFIC TESTS

##### • pH (791): 4.0–6.1

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
 USP Acetaminophen RS  
 USP Codeine Phosphate RS

### Acetaminophen and Codeine Phosphate Tablets

» Acetaminophen and Codeine Phosphate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

**Packaging and storage—**Preserve in tight, light-resistant containers, and store at controlled room temperature.

#### USP Reference standards (11)—

USP Acetaminophen RS

USP Codeine Phosphate RS

#### Identification—

**A:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** A quantity of finely powdered Tablets, equivalent to about 12 mg of codeine phosphate, meets the requirements of *Identification test B* under *Acetaminophen and Codeine Phosphate Capsules*.

#### Dissolution (711)—

*Medium:* 0.01 N hydrochloric acid; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

**Procedure—**Determine the amounts of acetaminophen ( $C_8H_9NO_2$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) dissolved by employing the procedure set forth in the *Assay*, except to use 0.01 N hydrochloric acid to prepare the *Codeine phosphate standard stock solution* and to make any other necessary volumetric adjustments.

**Tolerances—**Not less than 75% (Q) of the labeled amounts of  $C_8H_9NO_2$  and  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$  is dissolved in 30 minutes.

**Uniformity of dosage units (905):** meet the requirements.

#### PROCEDURE FOR CONTENT UNIFORMITY—

*Buffer solution, Mobile phase, Codeine phosphate standard stock solution, Standard preparation, and Chromatographic system—*Prepare as directed in the *Assay*.

**Sample preparation—**Transfer 1 Tablet to a 100-mL volumetric flask, add about 75 mL of *Mobile phase*, and sonicate for 10 minutes. Dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable 1- $\mu$ m filter.

**Procedure—**Separately inject equal volumes (about 30  $\mu$ L) of the *Standard preparation* and the *Sample preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in each Tablet taken by the formula:

$$1000C_A(r_U / r_S)$$

in which  $C_A$  is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Sample preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in each Tablet taken by the formula:

$$(406.37/397.37)1000C_C(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively;  $C_C$  is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; and the other terms are as defined herein.

#### Assay—

**Buffer solution—**Dissolve 2.04 g of monobasic potassium phosphate in about 950 mL of water. Add 2 mL of triethylamine, adjust with phosphoric acid to a pH of 2.35, dilute with water to 1000 mL, and mix.

**Mobile phase—**Prepare a filtered and degassed mixture of *Buffer solution* and methanol (92:8). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Codeine phosphate standard stock solution—**Dissolve an accurately weighed quantity of USP Codeine Phosphate RS



in *Mobile phase* to obtain a solution having a known concentration of about 0.3 mg per mL.

**Standard preparation**—Transfer about 30 mg of USP Acetaminophen RS and 100 J mL of Codeine phosphate standard stock solution, J being the ratio of the labeled amount, in mg, of codeine phosphate hemihydrate to that of acetaminophen, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.3 mg of acetaminophen and 0.3 J mg of codeine phosphate hemihydrate per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 300 mg of acetaminophen, to a 100-mL volumetric flask, add about 75 mL of *Mobile phase*, and sonicate for 10 minutes. Dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a suitable 1- $\mu$ m filter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between acetaminophen and codeine phosphate is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0% and 3.0%, respectively.

**Procedure**—Separately inject equal volumes (about 30  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken by the formula:

$$(LC_A / C_U)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of acetaminophen in each Tablet; *C<sub>A</sub>* is the concentration, in mg per mL, of USP Acetaminophen RS in the *Standard preparation*; *C<sub>U</sub>* is the concentration, in mg per mL, of acetaminophen in the *Assay preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Tablets taken by the formula:

$$(406.37/397.37)(LC_C / C_U)(r_U / r_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; *L* is the labeled quantity, in mg, of codeine phosphate hemihydrate in each Tablet; *C<sub>C</sub>* is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; *C<sub>U</sub>* is the concentration, in mg per mL, of codeine phosphate hemihydrate in the *Assay preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and the other terms are as defined herein.

## Acetaminophen, Dextromethorphan Hydrobromide, Doxylamine Succinate, and Pseudoephedrine Hydrochloride Oral Solution

### DEFINITION

Acetaminophen, Dextromethorphan Hydrobromide, Doxylamine Succinate, and Pseudoephedrine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of

the labeled amount of acetaminophen ( $C_8H_9NO_2$ ), dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ), doxylamine succinate ( $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ ), and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ).

### IDENTIFICATION

- **A.** The retention time of the acetaminophen peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay for Acetaminophen.
- **B.** The retention time of the dextromethorphan peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay for Dextromethorphan Hydrobromide.
- **C.** The retention time of the doxylamine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay for Doxylamine Succinate.
- **D.** The retention time of the pseudoephedrine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay for Pseudoephedrine Hydrochloride.

### ASSAY

#### Change to read:

#### • ACETAMINOPHEN

**Mobile phase:** Methanol and water (45:55)

**Standard solution:** 0.2 mg/mL of USP Acetaminophen RS in *Mobile phase*

**Sample solution:** Nominally 0.2 mg/mL of acetaminophen from a volume of Oral Solution in *Mobile phase* prepared as follows. Dilute a volume of Oral Solution, equivalent to about 200 mg of acetaminophen, in *Mobile phase*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

$\Delta$ USP40

**Tailing factor:** NMT 2.0 for the acetaminophen peak

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response of acetaminophen from the *Sample solution*

*r<sub>S</sub>* = peak response of acetaminophen from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ )

#### Change to read:

#### • DEXTROMETHORPHAN HYDROBROMIDE

**Solution A:** 6.8 g/L of monobasic potassium phosphate in water

**Mobile phase:** Acetonitrile and *Solution A* (45:55)

**Standard solution:** 0.1 mg/mL of USP Dextromethorphan Hydrobromide RS, 0.04 mg/mL of USP Doxyl-



amine Succinate RS, and 0.2 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Mobile phase*

**Sample solution:** Nominally 0.1 mg/mL of dextromethorphan hydrobromide from a volume of Oral Solution in *Mobile phase* prepared as follows. Dilute a volume of Oral Solution, equivalent to about 5 mg of dextromethorphan hydrobromide, in *Mobile phase*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L9

Flow rate: 2.5 mL/min

Injection volume: 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for pseudoephedrine, dextromethorphan, and doxylamine are 0.38, 0.65, and 1.0, respectively.]

#### Suitability requirements

▲<sub>USP40</sub>

**Tailing factor:** NMT 2.5 for the dextromethorphan, doxylamine, and pseudoephedrine peaks

**Relative standard deviation:** NMT 2.0% for dextromethorphan, doxylamine, and pseudoephedrine

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of dextromethorphan from the *Sample solution*

$r_S$  = peak response of dextromethorphan from the *Standard solution*

$C_S$  = concentration of USP Dextromethorphan Hydrobromide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of dextromethorphan hydrobromide in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of dextromethorphan hydrobromide monohydrate, ▲370.32▲<sub>USP40</sub>

$M_{r2}$  = molecular weight of anhydrous dextromethorphan hydrobromide, 352.32

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of dextromethorphan hydrobromide ( $C_{18}H_{25}NO \cdot HBr \cdot H_2O$ )

#### • DOXYLAMINE SUCCINATE

**Solution A, Mobile phase, Standard solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay for Dextromethorphan Hydrobromide*.

**Sample solution:** Nominally 0.04 mg/mL of doxylamine succinate from a volume of Oral Solution in *Mobile phase* prepared as follows. Dilute a volume of Oral Solution, equivalent to about 2 mg of doxylamine succinate, in *Mobile phase*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of doxylamine succinate ( $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of doxylamine from the *Sample solution*

$r_S$  = peak response of doxylamine from the *Standard solution*

$C_S$  = concentration of USP Doxylamine Succinate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of doxylamine succinate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of doxylamine succinate ( $C_{17}H_{22}N_2O \cdot C_4H_6O_4$ )

#### • PSEUDOEPHEDRINE HYDROCHLORIDE

**Solution A, Mobile phase, Standard solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay for Dextromethorphan Hydrobromide*.

**Sample solution:** Nominally 0.2 mg/mL of pseudoephedrine hydrochloride from a volume of Oral Solution in *Mobile phase* prepared as follows. Dilute a volume of Oral Solution, equivalent to about 10 mg of pseudoephedrine hydrochloride, in *Mobile phase*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of pseudoephedrine from the *Sample solution*

$r_S$  = peak response of pseudoephedrine from the *Standard solution*

$C_S$  = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ )

#### PERFORMANCE TESTS

##### • UNIFORMITY OF DOSAGE UNITS (905)

For single-unit containers

Acceptance criteria: Meets the requirements

##### • DELIVERABLE VOLUME (698)

For multiple-unit containers

Acceptance criteria: Meets the requirements

#### IMPURITIES

Add the following:

▲<sub>4</sub> 4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227): Meets the requirements▲<sub>USP40</sub>

#### SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total bacterial count does not exceed 100 cfu/g, the total combined molds and yeasts count does not exceed 10 cfu/g, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

• **pH (791):** 4.5–6.3

• **ALCOHOL DETERMINATION (611), Method II (if present):** 90.0%–110.0% of the labeled amount of alcohol ( $C_2H_5OH$ )

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.



- **USP REFERENCE STANDARDS (11)**
  - USP Acetaminophen RS
  - USP Dextromethorphan Hydrobromide RS
  - USP Doxylamine Succinate RS
  - USP Pseudoephedrine Hydrochloride RS

## Acetaminophen and Diphenhydramine Citrate Tablets

### DEFINITION

Acetaminophen and Diphenhydramine Citrate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and diphenhydramine citrate ( $C_{17}H_{21}NO \cdot C_6H_8O_7$ ).

### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution*, obtained in the *Assay for Acetaminophen* and in the *Assay for Diphenhydramine Citrate*, relative to the retention times of the respective internal standards, correspond to those of the respective *Standard solution*.

### ASSAY

#### Change to read:

#### • ACETAMINOPHEN

**Mobile phase:** Methanol and water (40:60)

**Diluent:** Methanol and water (1:4)

**Internal standard solution:** 8.0 mg/mL of guaifenesin in *Diluent*

**Standard stock solution:** 0.5 mg/mL of USP Acetaminophen RS, prepared as follows. Transfer 50 mg of USP Acetaminophen RS to a 100-mL volumetric flask. Dissolve in 2.5 mL of methanol, and dilute with water to volume.

**Standard solution:** 0.02 mg/mL of acetaminophen from *Standard stock solution* and 0.8 mg/mL of guaifenesin from *Internal standard solution*, in *Mobile phase*

**Sample stock solution:** Nominally 0.5 mg/mL of acetaminophen prepared as follows. Transfer a portion of the powder from NLT 20 finely powdered Tablets, nominally equivalent to an appropriate amount of acetaminophen, to a suitable volumetric flask. Add 25% of the total volume of methanol, and shake by mechanical means for 10 min. Dilute with water to volume.

**Sample solution:** Nominally 0.02 mg/mL of acetaminophen prepared as follows. Transfer 2.0 mL of *Sample stock solution* to a 50-mL volumetric flask, add 5.0 mL of *Internal standard solution*, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Column temperature:**  $35 \pm 0.5^\circ$

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for acetaminophen and guaifenesin are 0.5 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 6.0 between the analyte and internal standard peaks

<sup>▲</sup><sub>USP40</sub>

**Tailing factor:** NMT 2 for the analyte peak

**Relative standard deviation:** NMT 2.5% for the peak response ratios

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of acetaminophen to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of acetaminophen to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ )

#### Change to read:

#### • DIPHENHYDRAMINE CITRATE

**Diluent:** Methanol and water (1:1)

**Mobile phase:** Methanol, water, and glacial acetic acid (61:38:1) containing 1.0813 g of sodium 1-octanesulfonate in each 1000 mL of solution

**Internal standard solution:** 8 mg/mL of xylometazoline hydrochloride in water

**Standard solution:** 0.38 mg/mL of USP Diphenhydramine Citrate RS <sup>▲</sup> and 0.8 mg/mL of xylometazoline hydrochloride from *Internal standard solution* in *Diluent* <sup>▲</sup><sub>USP40</sub>

**Sample solution:** Nominally 0.38 mg/mL of diphenhydramine citrate prepared as follows. Transfer a portion of the powder from NLT 20 finely powdered Tablets, nominally equivalent to 38 mg of diphenhydramine citrate, to a 100-mL volumetric flask. Add 65 mL of *Diluent*, and shake by mechanical means for 15 min. Add 5.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Column temperature:**  $35 \pm 0.5^\circ$

**Flow rate:** 1.5 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for <sup>▲</sup><sub>USP40</sub> diphenhydramine citrate and xylometazoline hydrochloride are <sup>▲</sup><sub>USP40</sub> 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between the analyte and internal standard peaks

<sup>▲</sup><sub>USP40</sub>

**Tailing factor:** NMT 1.7 for the analyte peak

**Relative standard deviation:** NMT 2.0% for the peak response ratios

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of diphenhydramine citrate ( $C_{17}H_{21}NO \cdot C_6H_8O_7$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of diphenhydramine citrate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of diphenhydramine citrate to the internal standard from the *Standard solution*



- $C_S$  = concentration of USP Diphenhydramine Citrate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of diphenhydramine citrate in the *Sample solution* (mg/mL)  
**Acceptance criteria:** 90.0%–110.0% of the labeled amount of diphenhydramine citrate ( $C_{17}H_{21}NO \cdot C_6H_8O_7$ )

**PERFORMANCE TESTS**

- DISSOLUTION (711), Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms, Procedure for a pooled sample for immediate-release dosage forms**  
**Medium:** Water; 900 mL  
**Apparatus 2:** 50 rpm  
**Time:** 45 min  
**Analysis:** Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and diphenhydramine citrate ( $C_{17}H_{21}NO \cdot C_6H_8O_7$ ) dissolved using the procedures set forth in the *Assay for Acetaminophen* and the *Assay for Diphenhydramine Citrate*, respectively, and make any necessary volumetric adjustments.  
**Tolerances:** NLT 75% (Q) of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and diphenhydramine citrate ( $C_{17}H_{21}NO \cdot C_6H_8O_7$ ) is dissolved.
- UNIFORMITY OF DOSAGE UNITS (905), Content Uniformity:** Meet the requirements with respect to acetaminophen and diphenhydramine citrate

**IMPURITIES****Add the following:**

- 4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227):** Meet the requirements ▲USP40

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**  
 USP Acetaminophen RS  
 USP Diphenhydramine Citrate RS

## Acetaminophen, Diphenhydramine Hydrochloride, and Pseudoephedrine Hydrochloride Tablets

**DEFINITION**

Acetaminophen, Diphenhydramine Hydrochloride, and Pseudoephedrine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ), diphenhydramine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ), and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ).

**IDENTIFICATION**

- A.** The retention time of the acetaminophen peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Acetaminophen*.
- B.** The retention time of the diphenhydramine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Diphenhydramine Hydrochloride*.
- C.** The retention time of the pseudoephedrine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Pseudoephedrine Hydrochloride*.

**ASSAY****Change to read:****• ACETAMINOPHEN**

**Solution A:** Transfer 6.8 g of monobasic potassium phosphate to a 1000-mL volumetric flask, and add water to dissolve. Add 2.0 mL of triethylamine, and dilute with water to volume. Adjust with phosphoric acid to a pH of 4.0.

**Diluent:** Acetonitrile and *Solution A* (11:89)

**Mobile phase:** Acetonitrile and *Solution A* (6:94)

**Standard solution:** 25 µg/mL of USP Acetaminophen RS, 12.5 µg/mL of USP Diphenhydramine Hydrochloride RS, and 30 µg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*

**Sample stock solution:** Nominally 5 mg/mL of acetaminophen in *Diluent* prepared as follows. Transfer an amount nominally equivalent to 500 mg of acetaminophen from NLT 20 finely powdered Tablets to a 100-mL volumetric flask, add 75 mL of *Diluent*, shake, and sonicate for 15 min. Dilute with *Diluent* to volume.

**Sample solution:** Nominally 25 µg/mL of acetaminophen from the *Sample stock solution* in *Diluent*

**Chromatographic system**

(See *Chromatography (621), System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 15-cm; packing L10

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

▲USP40

**Tailing factor:** NMT 2.0 for the acetaminophen, diphenhydramine, and pseudoephedrine peaks

**Relative standard deviation:** NMT 2.0% determined from the acetaminophen, diphenhydramine hydrochloride, and pseudoephedrine hydrochloride responses for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of acetaminophen from the *Sample solution*

$r_S$  = peak response of acetaminophen from the *Standard solution*

$C_S$  = concentration of USP Acetaminophen RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of acetaminophen in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ )

**Change to read:****• DIPHENHYDRAMINE HYDROCHLORIDE**

**Solution A, Diluent, Mobile phase, Standard solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay for Acetaminophen*.

**Sample stock solution:** Nominally 0.125 mg/mL of diphenhydramine hydrochloride in *Diluent* prepared as follows. Transfer an amount nominally equivalent to 12.5 mg of diphenhydramine hydrochloride from a portion of finely powdered Tablets (NLT 20) to a 100-mL volumetric flask, add 75 mL of *Diluent*, and sonicate for 15 min. Dilute with *Diluent* to volume.



**Sample solution:** Nominally 12.5 µg/mL of diphenhydramine hydrochloride  $\Delta_{\text{USP40}}$  from the *Sample stock solution* in *Diluent*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of diphenhydramine hydrochloride ( $\text{C}_{17}\text{H}_{21}\text{NO} \cdot \text{HCl}$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of diphenhydramine from the *Sample solution*

$r_S$  = peak response of diphenhydramine from the *Standard solution*

$C_S$  = concentration of USP Diphenhydramine Hydrochloride RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of diphenhydramine hydrochloride in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of diphenhydramine hydrochloride ( $\text{C}_{17}\text{H}_{21}\text{NO} \cdot \text{HCl}$ )

#### • PSEUDOEPHEDRINE HYDROCHLORIDE

**Solution A, Diluent, Mobile phase, Standard solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay for Acetaminophen*.

**Sample stock solution:** Nominally 0.3 mg/mL of pseudoephedrine hydrochloride in *Diluent* prepared as follows. Transfer an amount nominally equivalent to 30 mg of pseudoephedrine hydrochloride from a portion of finely powdered Tablets (NLT 20) to a 100-mL volumetric flask, add 75 mL of *Diluent*, and sonicate for 15 min. Dilute with *Diluent* to volume.

**Sample solution:** Nominally 30 µg/mL of pseudoephedrine hydrochloride from the *Sample stock solution* in *Diluent*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of pseudoephedrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO} \cdot \text{HCl}$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of pseudoephedrine from the *Sample solution*

$r_S$  = peak response of pseudoephedrine from the *Standard solution*

$C_S$  = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of pseudoephedrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO} \cdot \text{HCl}$ )

#### PERFORMANCE TESTS

- **DISSOLUTION** (711), *Procedure*, *Apparatus 1* and *Apparatus 2*, *Immediate-Release Dosage Forms*, *Procedure for a pooled sample for immediate-release dosage forms*

**Medium:** pH 5.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Solution A, Diluent, Mobile phase, Standard solution, and Chromatographic system:** Proceed as directed in the *Assay for Acetaminophen*.

**Sample solution A:** Combine equal volumes of the filtered solutions, and use the pooled sample.

**Sample solution B:** Transfer 5.0 mL of *Sample solution A* to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume.

**Analysis:** Using *Sample solution A* and the *Standard solution*, and making any necessary volumetric adjustments, proceed as directed in the *Assay for Diphenhydramine Hydrochloride* and the *Assay for Pseudoephedrine Hydrochloride*, and determine the percentage of the labeled amount of diphenhydramine hydrochloride ( $\text{C}_{17}\text{H}_{21}\text{NO} \cdot \text{HCl}$ ) and pseudoephedrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO} \cdot \text{HCl}$ ) dissolved. Using *Sample solution B* and the *Standard solution*, and making any necessary volumetric adjustments, proceed as directed in the *Assay for Acetaminophen*, and determine the percentage of the labeled amount of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ) dissolved.

**Tolerances:** NLT 75% (Q) of the labeled amount of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ), diphenhydramine hydrochloride ( $\text{C}_{17}\text{H}_{21}\text{NO} \cdot \text{HCl}$ ), and pseudoephedrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO} \cdot \text{HCl}$ ) is dissolved.

#### For Tablets labeled as chewable

**Medium:** pH 5.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

**Apparatus 2:** 75 rpm

**Time:** 45 min

**Tolerances:** NLT 75% (Q) of the labeled amount of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ), diphenhydramine hydrochloride ( $\text{C}_{17}\text{H}_{21}\text{NO} \cdot \text{HCl}$ ), and pseudoephedrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO} \cdot \text{HCl}$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### IMPURITIES

##### Add the following:

- **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** (227): Meet the requirements  $\Delta_{\text{USP40}}$

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Acetaminophen RS  
USP Diphenhydramine Hydrochloride RS  
USP Pseudoephedrine Hydrochloride RS

## Acetaminophen and Pseudoephedrine Hydrochloride Tablets

#### DEFINITION

Acetaminophen and Pseudoephedrine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ) and pseudoephedrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO} \cdot \text{HCl}$ ).

#### IDENTIFICATION

- **A.** The retention times of the acetaminophen and pseudoephedrine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### Change to read:

#### • PROCEDURE

**Diluent:** Acetonitrile and water (10:90)

**Solution A:** 0.005 M ethanesulfonic acid and 0.05 M monobasic potassium phosphate

**Mobile phase:** Acetonitrile and *Solution A* (100:900). Adjust with 5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.6.

**Pseudoephedrine hydrochloride standard stock solution:** 0.6 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*



**Standard solution:** Transfer 6J mg of USP Acetaminophen RS to a 100-mL volumetric flask, J being the ratio of the labeled quantity (mg) of acetaminophen to the labeled quantity (mg) of pseudoephedrine hydrochloride in each Tablet. Add 2.0 mL of 1 N hydrochloric acid and 20 mL of *Diluent*, and mix to dissolve. Add 10.0 mL of *Pseudoephedrine hydrochloride standard solution* and dilute with *Diluent* to volume. This solution contains 0.06J mg/mL of USP Acetaminophen RS and 0.06 mg/mL of USP Pseudoephedrine Hydrochloride RS.

**Sample solution:** Nominally 0.06 mg/mL of pseudoephedrine hydrochloride prepared as follows. Transfer a portion of finely powdered Tablets (NLT 20), equivalent to 30 mg of pseudoephedrine hydrochloride, to a 500-mL volumetric flask, add 10.0 mL of 1 N hydrochloric acid and 100 mL of *Diluent*, and sonicate for 30 min, with occasional shaking. Allow to cool, and dilute with *Diluent* to volume. Pass a portion of this solution through a glass fiber filter, and use the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm × 25-cm; base-deactivated or end-capped packing L1

**Flow rate:** 3 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for acetaminophen and pseudoephedrine are about 0.55 and 1.0, respectively. ▲<sub>USP40</sub>]

#### Suitability requirements

**Resolution:** NLT 3.5 between acetaminophen and pseudoephedrine

**Tailing factor:** NMT 2 for the pseudoephedrine peak

**Relative standard deviation:** NMT 2.0% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the corresponding analyte from the *Sample solution*

$r_S$  = peak response of the corresponding analyte from the *Standard solution*

$C_S$  = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the appropriate analyte in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ )

#### PERFORMANCE TESTS

- **DISSOLUTION (711), Procedure, Apparatus 1 and Apparatus 2, Immediate-Release Dosage Forms, Procedure for a pooled sample for immediate-release dosage forms**

**Medium:** pH 5.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

Determine the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) dissolved by using the following method.

**Mobile phase:** Proceed as directed in the Assay.

**Standard solution:** (L/900) mg/mL of USP Pseudoephedrine Hydrochloride RS and (L/900) mg/mL of USP

Acetaminophen RS in *Medium*. [NOTE—L is the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Tablet; and J is the ratio of the labeled quantity, in mg, of acetaminophen to the labeled quantity, in mg, of pseudoephedrine hydrochloride in each Tablet.]

**Sample solution:** Filtered portion of the solution under test, suitably diluted with *Medium*, if necessary

**Chromatographic system and System suitability:** Proceed as directed in the Assay, except to inject the *Standard solution*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Inject 20 µL of the *Samples*, and measure the responses for the acetaminophen and pseudoephedrine peaks.]

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times V \times (C_S/L) \times 100$$

$r_U$  = peak response of the corresponding analyte from the *Sample solution*

$r_S$  = peak response of the corresponding analyte from the *Standard solution*

V = volume of *Medium*, 900 mL

$C_S$  = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

L = label amount of the corresponding analyte in a Tablet (mg)

**Tolerances:** NLT 75% (Q) of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) is dissolved.

#### For Tablets labeled as chewable

**Medium:** pH 5.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

**Apparatus 2:** 75 rpm

**Time:** 45 min

**Standard solution, Sample solution, Chromatographic system, System suitability, and Analysis:** Proceed as directed above in *Procedure for a pooled sample for immediate-release dosage forms*.

**Tolerances:** NLT 75% (Q) of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### Add the following:

- ▲ 4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227): Meet the requirements ▲<sub>USP40</sub>

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Acetaminophen RS  
USP Pseudoephedrine Hydrochloride RS

## Acetaminophen and Tramadol Hydrochloride Tablets

**New title:** *Tramadol Hydrochloride and Acetaminophen Tablets*

(Title for this monograph—not to change until February 1, 2017)

(Prior to February 1, 2017, the current practice of labeling the article of commerce with the name Acetaminophen



and Tramadol Hydrochloride Tablets may be continued. Use of the name Tramadol Hydrochloride and Acetaminophen Tablets will be permitted as of August 1, 2014, but the use of this name will not be mandatory until February 1, 2017. The 30-month extension will provide the time needed by manufacturers and users to make necessary changes.)

#### DEFINITION

Acetaminophen and Tramadol Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ ) and acetaminophen ( $C_8H_9NO_2$ ).

#### IDENTIFICATION

- A. The retention times of Tramadol sample solution and the Acetaminophen sample solution correspond to those of the Standard solution, as obtained in the Assay.

#### ASSAY

##### Change to read:

#### PROCEDURE

**Mobile phase:** Tetrahydrofuran, triethylamine, water, and trifluoroacetic acid (8: 0.1: 92: 0.1). The apparent pH of the final solvent mixture should be between 2.2 and 2.4.

**Diluent:** Methanol and water (1:9)

**Standard solution:** 0.065 mg/mL of USP Acetaminophen RS and 0.075 mg/mL of USP Tramadol Hydrochloride RS in Diluent. Sonication may be used to aid dissolution.

**Sample stock solution:** Weigh NLT 20 Tablets, and determine the average Tablet weight. Grind the Tablets into a fine powder, and transfer an amount equivalent to one Tablet to a 50-mL volumetric flask. Add 30 mL of Diluent with continuous shaking to disperse the powder. Sonicate for 15 min with intermittent shaking, and shake the flask on a mechanical shaker for 30 min. Dilute with Diluent to volume, and mix well. Centrifuge the suspension, and use the supernatant for subsequent dilutions.

**Tramadol sample solution:** Nominally 75 µg/mL of tramadol hydrochloride in Diluent from the Sample stock solution

**Acetaminophen sample solution:** Nominally 65 µg/mL of acetaminophen in Diluent from the Sample stock solution

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 216 nm for tramadol hydrochloride and UV 249 nm for acetaminophen

**Column:** 4.6-mm × 15-cm; 5-µm packing L11

**Column temperature:** 50°

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**Run time:** 4 times the retention time of acetaminophen

#### System suitability

**Sample:** Standard solution

#### Suitability requirements

**Resolution:** NLT 10.0 between acetaminophen and tramadol

▲ USP40

**Tailing factor:** NMT 2.0 for each analyte

**Relative standard deviation:** NMT 2.0% for each analyte

#### Analysis

**Samples:** Standard solution, Tramadol sample solution, and Acetaminophen sample solution

Calculate the percentage of the labeled amount of tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of tramadol from the Tramadol sample solution

$r_S$  = peak response of tramadol from the Standard solution

$C_S$  = concentration of USP Tramadol Hydrochloride RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of tramadol hydrochloride in the Tramadol sample solution (mg/mL)

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of acetaminophen from the Acetaminophen sample solution

$r_S$  = peak response of acetaminophen from the Standard solution

$C_S$  = concentration of USP Acetaminophen RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of acetaminophen in the Acetaminophen sample solution (mg/mL)

#### Acceptance criteria

**Tramadol hydrochloride:** 90.0%–110.0% of the labeled amount of tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ )

**Acetaminophen:** 90.0%–110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ )

#### PERFORMANCE TESTS

##### DISSOLUTION (711)

###### Test 1

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Buffer solution:** 6.8 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.50.

**Mobile phase:** Acetonitrile and Buffer solution (1:4)

**Standard solution:** 0.36 mg/mL of USP Acetaminophen RS and 0.04 mg/mL of USP Tramadol Hydrochloride RS in Medium

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 272 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L7

**Column temperature:** 25°

**Flow rate:** 1 mL/min

**Injection volume:** 25 µL

**Run time:** 2 times the retention time of tramadol

#### System suitability

**Sample:** Standard solution

[NOTE—The relative retention times for acetaminophen and tramadol are about 0.5 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 5.0 between the acetaminophen and tramadol peaks

**Relative standard deviation:** NMT 2.0% for both the acetaminophen and tramadol peaks



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times (1/L) \times 100$$

$r_u$  = peak response of acetaminophen or tramadol from the *Sample solution*

$r_s$  = peak response of acetaminophen or tramadol from the *Standard solution*

$C_s$  = concentration of USP Acetaminophen RS or USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim for acetaminophen or tramadol hydrochloride (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 20 min

**Buffer solution, Mobile phase, Standard solution, Sample solution, Chromatographic system, and Analysis:** Proceed as directed in *Dissolution Test 1*.

**Tolerances:** NLT 80% (Q) of the labeled amounts of acetaminophen ( $C_8H_9NO_2$ ) and tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**OTHER COMPONENTS****Delete the following:**▲ **LIMIT OF p-AMINOPHENOL**

All Standards, the *Sample solution*, and the *Blank solution* must be mixed with the *Basic ferricyanide solution* and analyzed as soon as possible after a 30-min waiting period.

**Diluent:** Methanol and water (1:1)

**Basic ferricyanide solution:** Dissolve 1 g of sodium nitroferricyanide and 1 g of anhydrous sodium carbonate in 100 mL of water.

**Standard solution:** Dissolve USP *p*-Aminophenol RS in *Diluent* to obtain a solution having a known concentration of 0.05 mg/mL. Sonicate if necessary to dissolve. Transfer 5 mL of the resulting solution to a 100-mL volumetric flask, and add 50 mL of *Diluent* and 5 mL of *Basic ferricyanide solution*. Dilute with *Diluent* to volume, and mix. Let stand for 30 min. Pass the solution through a nylon membrane filter of 0.45- $\mu$ m pore size, and use the filtrate.

**Sample solution:** Weigh NLT 20 Tablets. Grind the Tablets into a fine powder. Accurately transfer an amount of powder, equivalent to about 5 g of acetaminophen based on the label claim, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and sonicate for 15 min with intermittent shaking, followed by mechanical shaking for 30 min. Add 6 mL of *Basic ferricyanide solution*. Dilute with *Diluent* to volume, mix, and let stand for 30 min. Centrifuge a portion of the solution, pass the clear supernatant through a nylon membrane filter of 0.45- $\mu$ m pore size, and use the filtrate for analysis.

**Blank solution:** Add 50 mL of *Diluent* to a 100-mL volumetric flask. Add 5 mL of *Basic ferricyanide solution*. Dilute with *Diluent* to volume, and let stand for 30 min. Pass a portion of the solution through a nylon membrane filter of 0.45- $\mu$ m pore size, and use the filtrate for analysis.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 710 nm

**Cell:** 1 cm

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 6.0%. The percent difference between the initial and final absorbance readings of the *Standard solution* differs by NMT 10%.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of *p*-aminophenol ( $C_6H_7NO$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP *p*-Aminophenol RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.01%▲USP40

**IMPURITIES****Add the following:**

- ▲ **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS (227):** Meet the requirements▲USP40

**Change to read:**• **ORGANIC IMPURITIES**

**Mobile phase, Diluent, and Sample stock solution:** Proceed as directed in the *Assay*.

**Standard solution:** 0.75  $\mu$ g/mL each of USP Tramadol Hydrochloride RS and USP Tramadol Related Compound A RS in *Diluent*

**Sample solution:** Pass a suitable volume of *Sample stock solution* through a nylon membrane filter of 0.45- $\mu$ m pore size. Use the filtrate after discarding the first 4 mL of filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** 216 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L11

**Column temperature:** 50°

**Flow rate:** 1 mL/min

**Injection volume:** 30  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between tramadol related compound A and tramadol hydrochloride

▲USP40

**Relative standard deviation:** NMT 6.0% for tramadol hydrochloride

**Analysis**

**Samples:** *Diluent*, *Standard solution*, and *Sample solution*. Disregard the peaks due to the *Diluent*.

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of each degradation product from the *Sample solution*

$r_s$  = peak response of tramadol from the *Standard solution*



- $C_s$  = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_u$  = nominal concentration of tramadol hydrochloride in the *Sample solution* ( $\mu\text{g/mL}$ )  
 Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
O-Desmethyl-tramadol <sup>a</sup>	0.60	0.2
Tramadol related compound A	0.80	0.2
Tramadol	1.0	—
Acetaminophen	0.38	—
Any other individual, unspecified degradation product	—	0.2
Total degradation products	—	0.8

<sup>a</sup> 3-[(1*S*,2*R*)-2-[(Dimethylamino)methyl]-1-hydroxycyclohexyl]phenol.

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

#### Change to read:

#### • USP REFERENCE STANDARDS (11)

USP Acetaminophen RS

▲USP 4-Aminophenol RS<sub>▲USP40</sub>

4-Amino-1-hydroxybenzene.

$\text{C}_6\text{H}_7\text{NO}$  109.13

USP Tramadol Hydrochloride RS

(±)-*cis*-2-[(Dimethylamino)methyl]-1-(*m*-methoxyphenyl)cyclohexanol hydrochloride.

$\text{C}_{16}\text{H}_{25}\text{NO}_2 \cdot \text{HCl}$  299.84

USP Tramadol Related Compound A RS

*RS,SR*-1-(3-Methoxyphenyl)-2-(dimethylaminomethyl)cyclohexanol hydrochloride.

## Tramadol Hydrochloride and Acetaminophen Tablets

Former title: *Acetaminophen and Tramadol Hydrochloride Tablets*

(Title for this monograph—not to change until February 1, 2017)

(Prior to February 1, 2017, the current practice of labeling the article of commerce with the name *Acetaminophen and Tramadol Hydrochloride Tablets* may be continued. Use of the name *Tramadol Hydrochloride and Acetaminophen Tablets* will be permitted as of August 1, 2014, but the use of this name will not be mandatory until February 1, 2017. The 30-month extension will provide the time needed by manufacturers and users to make necessary changes.)

#### DEFINITION

Tramadol Hydrochloride and Acetaminophen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of tramadol hydrochloride ( $\text{C}_{16}\text{H}_{25}\text{NO}_2 \cdot \text{HCl}$ ) and acetaminophen ( $\text{C}_8\text{H}_9\text{NO}_2$ ).

#### IDENTIFICATION

- A.** The retention times of the *Tramadol sample solution* and the *Acetaminophen sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

#### Change to read:

#### • PROCEDURE

**Mobile phase:** Tetrahydrofuran, triethylamine, water, and trifluoroacetic acid (8:0.1:92:0.1). The apparent pH of the final solvent mixture should be between 2.2 and 2.4.

**Diluent:** Methanol and water (1:9)

**Standard solution:** 0.065 mg/mL of USP Acetaminophen RS and 0.075 mg/mL of USP Tramadol Hydrochloride RS in *Diluent*. Sonication may be used to aid dissolution.

**Sample stock solution:** Weigh NLT 20 Tablets, and determine the average Tablet weight. Grind the Tablets into a fine powder, and transfer an amount equivalent to one Tablet to a 50-mL volumetric flask. Add 30 mL of *Diluent* with continuous shaking to disperse the powder. Sonicate for 15 min with intermittent shaking, and shake the flask on a mechanical shaker for 30 min. Dilute with *Diluent* to volume, and mix well. Centrifuge the suspension, and use the supernatant for subsequent dilutions.

**Tramadol sample solution:** Nominally 75  $\mu\text{g/mL}$  of tramadol hydrochloride in *Diluent* from the *Sample stock solution*

**Acetaminophen sample solution:** Nominally 65  $\mu\text{g/mL}$  of acetaminophen in *Diluent* from the *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 216 nm for tramadol hydrochloride and UV 249 nm for acetaminophen

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu\text{m}$  packing L11

**Column temperature:** 50°

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu\text{L}$

**Run time:** 4 times the retention time of acetaminophen

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 10.0 between acetaminophen and tramadol

▲▲USP40

**Tailing factor:** NMT 2.0 for each analyte

**Relative standard deviation:** NMT 2.0% for each analyte

#### Analysis

**Samples:** *Standard solution*, *Tramadol sample solution*, and *Acetaminophen sample solution*

Calculate the percentage of the labeled amount of tramadol hydrochloride ( $\text{C}_{16}\text{H}_{25}\text{NO}_2 \cdot \text{HCl}$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of tramadol from the *Tramadol sample solution*

$r_s$  = peak response of tramadol from the *Standard solution*

$C_s$  = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of tramadol hydrochloride in the *Tramadol sample solution* (mg/mL)



Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of acetaminophen from the *Acetaminophen sample solution*  
 $r_S$  = peak response of acetaminophen from the *Standard solution*  
 $C_S$  = concentration of USP Acetaminophen RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of acetaminophen in the *Acetaminophen sample solution* (mg/mL)

#### Acceptance criteria

**Tramadol hydrochloride:** 90.0%–110.0% of the labeled amount of tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ )

**Acetaminophen:** 90.0%–110.0% of the labeled amount of acetaminophen ( $C_8H_9NO_2$ )

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

###### Test 1

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Buffer solution:** 6.8 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.50.

**Mobile phase:** Acetonitrile and *Buffer solution* (1:4)

**Standard solution:** 0.36 mg/mL of USP Acetaminophen RS and 0.04 mg/mL of USP Tramadol Hydrochloride RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 272 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7

**Column temperature:** 25°

**Flow rate:** 1 mL/min

**Injection volume:** 25  $\mu$ L

**Run time:** 2 times the retention time of tramadol

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for acetaminophen and tramadol are about 0.5 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 5.0 between the acetaminophen and tramadol peaks

**Relative standard deviation:** NMT 2.0% for both the acetaminophen and tramadol peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

- $r_U$  = peak response of acetaminophen or tramadol from the *Sample solution*  
 $r_S$  = peak response of acetaminophen or tramadol from the *Standard solution*  
 $C_S$  = concentration of USP Acetaminophen RS or USP Tramadol Hydrochloride RS in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 900 mL  
 $L$  = label claim for acetaminophen or tramadol hydrochloride (mg/Tablet)  
**Tolerances:** NLT 80% (Q) of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 20 min

**Buffer solution, Mobile phase, Standard solution, Sample solution, Chromatographic system, System suitability, and Analysis:** Proceed as directed in *Dissolution Test 1*.

**Tolerances:** NLT 80% (Q) of the labeled amount of acetaminophen ( $C_8H_9NO_2$ ) and tramadol hydrochloride ( $C_{16}H_{25}NO_2 \cdot HCl$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### OTHER COMPONENTS

##### Delete the following:

##### • LIMIT OF *p*-AMINOPHENOL

All Standards, the *Sample solution*, and the *Blank solution* must be mixed with the *Basic ferricyanide solution* and analyzed as soon as possible after a 30-min waiting period.

**Diluent:** Methanol and water (1:1)

**Basic ferricyanide solution:** Dissolve 1 g of sodium nitroferricyanide and 1 g of anhydrous sodium carbonate in 100 mL of water.

**Standard solution:** Dissolve USP *p*-Aminophenol RS in *Diluent* to obtain a solution having a known concentration of 0.05 mg/mL. Sonicate if necessary to dissolve. Transfer 5 mL of the resulting solution to a 100-mL volumetric flask, and add 50 mL of *Diluent* and 5 mL of *Basic ferricyanide solution*. Dilute with *Diluent* to volume, and mix. Let stand for 30 min. Pass the solution through a nylon membrane filter of 0.45- $\mu$ m pore size, and use the filtrate.

**Sample solution:** Weigh NLT 20 Tablets. Grind the Tablets into a fine powder. Accurately transfer an amount of powder, equivalent to about 5 g of acetaminophen based on the label claim, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and sonicate for 15 min with intermittent shaking, followed by mechanical shaking for 30 min. Add 6 mL of *Basic ferricyanide solution*. Dilute with *Diluent* to volume, mix, and let stand for 30 min. Centrifuge a portion of the solution, pass the clear supernatant through a nylon membrane filter of 0.45- $\mu$ m pore size, and use the filtrate for analysis.

**Blank solution:** Add 50 mL of *Diluent* to a 100-mL volumetric flask. Add 5 mL of *Basic ferricyanide solution*. Dilute with *Diluent* to volume, and let stand for 30 min. Pass a portion of the solution through a nylon membrane filter of 0.45- $\mu$ m pore size, and use the filtrate for analysis.

#### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 710 nm

**Cell:** 1 cm

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 6.0%. The percentage difference between the initial and final absorbance readings of the *Standard solution* differs by NMT 10%.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of *p*-aminophenol ( $C_6H_7NO$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*



- $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP *p*-Aminophenol RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of acetaminophen in the *Sample solution* (mg/mL)  
 Acceptance criteria: NMT 0.01%<sup>▲USP40</sup>

**IMPURITIES****Add the following:**

- ▲ **4-AMINOPHENOL IN ACETAMINOPHEN-CONTAINING DRUG PRODUCTS** (227): Meet the requirements<sup>▲USP40</sup>

**Change to read:**• **ORGANIC IMPURITIES**

Mobile phase, Diluent, and Sample stock solution: Proceed as directed in the *Assay*.

**Standard solution:** 0.75 µg/mL each of USP Tramadol Hydrochloride RS and USP Tramadol Related Compound A RS in *Diluent*

**Sample solution:** Pass a suitable volume of *Sample stock solution* through a nylon membrane filter of 0.45-µm pore size. Use the filtrate after discarding the first 4 mL of filtrate.

**Chromatographic system**  
 (See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: 216 nm

Column: 4.6-mm × 15-cm; 5-µm packing L11

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 30 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between tramadol related compound A and tramadol hydrochloride

▲<sup>USP40</sup>

**Relative standard deviation:** NMT 6.0% for tramadol hydrochloride

**Analysis**

**Samples:** *Diluent*, *Standard solution*, and *Sample solution*. Disregard the peaks due to the *Diluent*.

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of each degradation product from the *Sample solution*  
 $r_s$  = peak response of tramadol from the *Standard solution*  
 $C_s$  = concentration of USP Tramadol Hydrochloride RS in the *Standard solution* (µg/mL)  
 $C_u$  = nominal concentration of tramadol hydrochloride in the *Sample solution* (µg/mL)  
 Acceptance criteria: See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
O-Desmethyl-tramadol <sup>a</sup>	0.60	0.2
Tramadol related compound A	0.80	0.2
Tramadol	1.0	—
Acetaminophen	0.38	—

<sup>a</sup> 3-[(1*RS*,2*RS*)-2-[(Dimethylamino)methyl]-1-hydroxycyclohexyl]phenol.

**Table 1** (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual, unspecified degradation product	—	0.2
Total degradation products	—	0.8

<sup>a</sup> 3-[(1*RS*,2*RS*)-2-[(Dimethylamino)methyl]-1-hydroxycyclohexyl]phenol.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

**Change to read:**• **USP REFERENCE STANDARDS** (11)

USP Acetaminophen RS

▲USP 4-Aminophenol RS<sup>▲USP40</sup>

4-Amino-1-hydroxybenzene.

C<sub>6</sub>H<sub>7</sub>NO 109.13

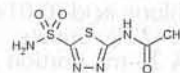
USP Tramadol Hydrochloride RS

(±)-*cis*-2-[(Dimethylamino)methyl]-1-(*m*-methoxyphenyl)cyclohexanol hydrochloride.

C<sub>16</sub>H<sub>25</sub>NO<sub>2</sub> · HCl 299.84

USP Tramadol Related Compound A RS

RS,SR-1-(3-Methoxyphenyl)-2-(dimethylaminomethyl)cyclohexanol hydrochloride.

**Acetazolamide**

C<sub>4</sub>H<sub>6</sub>N<sub>4</sub>O<sub>3</sub>S<sub>2</sub> 222.25  
 Acetamide, N-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]-;  
 N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide [59-66-5].

**DEFINITION**

Acetazolamide contains NLT 98.0% and NMT 102.0% of acetazolamide (C<sub>4</sub>H<sub>6</sub>N<sub>4</sub>O<sub>3</sub>S<sub>2</sub>), calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, add 20 mL of methanol and 30 mL of acetonitrile, and mix. Adjust with glacial acetic acid to a pH of 4.0.

**Standard solution:** 0.1 mg/mL of USP Acetazolamide RS prepared as follows. Transfer USP Acetazolamide RS into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.

**Sample solution:** 0.1 mg/mL of Acetazolamide prepared as follows. Transfer Acetazolamide into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

**System suitability**Sample: *Standard solution***Suitability requirements**

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of acetazolamide (C<sub>4</sub>H<sub>6</sub>N<sub>4</sub>O<sub>3</sub>S<sub>2</sub>) in the portion of Acetazolamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of acetazolamide from the *Sample solution*

$r_S$  = peak response of acetazolamide from the *Standard solution*

$C_S$  = concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Acetazolamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

- **CHLORIDE AND SULFATE** (221), *Chloride*

Sample solution: Digest 1.5 g with 75 mL of water at about 70° for 5 min. Cool to room temperature, and filter.

Acceptance criteria: A 25-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).

- **CHLORIDE AND SULFATE** (221), *Sulfate*

Sample solution: A 25-mL portion of the filtrate prepared in the test for *Chloride*

Acceptance criteria: It shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.04%).

- **SELENIUM** (291)

Sample: 200 mg

Acceptance criteria: NMT 30 ppm

**Delete the following:**

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm (Official 1-

Jan-2018)

- **SILVER-REDUCING SUBSTANCES**

Sample: 5 g

Analysis: Thoroughly wet the *Sample* with alcohol. Add 125 mL of water, 10 mL of nitric acid, and 5.0 mL of 0.1 N silver nitrate VS. Stir with a mechanical stirrer for 30 min. Filter, add 5 mL of ferric ammonium sulfate TS to the filtrate, and titrate with 0.1 N ammonium thiocyanate VS to a reddish-brown endpoint.

Acceptance criteria: NLT 4.8 mL of 0.1 N ammonium thiocyanate is required.

- **ORGANIC IMPURITIES**

Procedure: *Ordinary Impurities* (466)

Standard solution: Acetone and methanol (1:1)

Test solution: Acetone and methanol (1:1)

Eluant: *n*-Propyl alcohol and 1 N ammonium hydroxide (88:12)

Visualization: 1

**SPECIFIC TESTS**

- **WATER DETERMINATION** (921), *Method I*: NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight containers, and store at room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Acetazolamide RS

**Acetazolamide for Injection****DEFINITION**

Acetazolamide for Injection is prepared from Acetazolamide with the aid of sodium hydroxide. It is suitable for parenteral use. The contents of each container, when constituted as directed in the labeling, yield a solution containing NLT 95.0% and NMT 110.0% of the labeled amount of acetazolamide (C<sub>4</sub>H<sub>6</sub>N<sub>4</sub>O<sub>3</sub>S<sub>2</sub>).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)

Sample: Dissolve 500 mg in 5 mL of water, add 2 drops of hydrochloric acid, and allow the mixture to stand for about 15 min. Filter through a fine sintered-glass funnel, wash with several small portions of water, and dry under vacuum over silica gel for 3 h.

Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- **C. IDENTIFICATION TESTS—GENERAL** (191), *Sodium*: Meets the requirements

**ASSAY**

- **PROCEDURE**

Mobile phase: Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, add 20 mL of methanol and 30 mL of acetonitrile, and mix. Adjust with glacial acetic acid to a pH of 4.0.

Standard solution: 0.1 mg/mL of USP Acetazolamide RS prepared as follows. Transfer USP Acetazolamide RS into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.

Sample solution: Nominally equivalent to 0.1 mg/mL of acetazolamide prepared as follows. Dissolve the contents of 1 container of Acetazolamide for Injection in a measured volume of water corresponding to the volume of solvent specified in the labeling. Dilute a portion of this solution quantitatively and stepwise with water.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

**System suitability**Sample: *Standard solution***Suitability requirements**

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetazolamide (C<sub>4</sub>H<sub>6</sub>N<sub>4</sub>O<sub>3</sub>S<sub>2</sub>) in the portion of Acetazolamide for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response of acetazolamide from the *Sample solution*  
 $r_S$  = peak response of acetazolamide from the *Standard solution*  
 $C_S$  = concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of acetazolamide in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–110.0%

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

#### SPECIFIC TESTS

- **PH** (791): 9.0–10.0, in a freshly prepared solution (1 in 10)
- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.5 USP Endotoxin Units/mg of acetazolamide.
- **LABELING** (7), *Labels and Labeling for Injectable Products*: Meets the requirements
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** (1), *Specific Tests, Completeness and clarity of solutions*: Meets the requirements at the time of use
- **STERILITY TESTS** (71): Meets the requirements

#### ADDITIONAL REQUIREMENTS

##### Change to read:

- **PACKAGING AND STORAGE**: Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017), preferably of Type III glass, and store at room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Acetazolamide RS  
USP Endotoxin RS

## Acetazolamide Compounded Oral Suspension

#### DEFINITION

Acetazolamide Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of acetazolamide ( $C_4H_6N_4O_3S_2$ ).

Prepare Acetazolamide Compounded Oral Suspension, 25 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Acetazolamide	2.5 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, NF (regular or sugar-free), and Vehicle for Oral Suspension, NF, or Cherry Syrup, NF, a sufficient quantity to make	100 mL

If using tablets, place in a mortar and comminute to a fine powder, or add *Acetazolamide* powder. Add about 20 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough liquid *Vehicle* to bring to final volume, and mix well.

#### ASSAY

##### PROCEDURE

**Mobile phase:** Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, and add 20 mL of methanol and 30 mL of acetonitrile. Adjust with glacial acetic acid to a pH of 4.0.

**Standard stock solution:** Transfer about 25 mg of USP Acetazolamide RS, accurately weighed, to a 50-mL volumetric flask, add 5.0 mL of 0.5 N sodium hydroxide, and mix to dissolve. Dilute with water to volume, and mix.

**Standard solution:** 250 µg/mL of USP Acetazolamide RS from the *Standard stock solution* in water

**Sample solution:** 250 µg/mL of acetazolamide from Oral Suspension in *Mobile phase*. Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at  $-70^\circ$  until analyzed. At the time of analysis, remove the sample from the freezer, allow to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; 5-µm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for the acetazolamide peak is about 3 min.]

#### Suitability requirements

**Relative standard deviation:** NMT 1.1% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetazolamide ( $C_4H_6N_4O_3S_2$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Acetazolamide RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of acetazolamide in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

#### SPECIFIC TESTS

- **PH** (791): 4.0–5.0 (Vehicle for Oral Solution and Vehicle for Oral Suspension), 3.1–3.9 (Cherry Syrup)

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded when stored at controlled room temperature, or in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** (11)  
USP Acetazolamide RS

## Acetazolamide Tablets

#### DEFINITION

Acetazolamide Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of acetazolamide ( $C_4H_6N_4O_3S_2$ ).



**IDENTIFICATION**• **A. INFRARED ABSORPTION (197K)**

**Sample:** Extract a quantity of finely powdered Tablets, equivalent to about 500 mg of acetazolamide, with 50 mL of acetone. Filter, and add sufficient solvent hexane to the filtrate to cause formation of a heavy, white precipitate. Collect the precipitate on a medium-porosity, sintered-glass funnel, and dry with suction.

**Acceptance criteria:** Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water, add 20 mL of methanol and 30 mL of acetonitrile, and mix. Adjust with glacial acetic acid to a pH of 4.0.

**Standard solution:** 0.1 mg/mL of USP Acetazolamide RS prepared as follows. Transfer USP Acetazolamide RS into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the final volume, and dilute with water to volume.

**Sample stock solution:** Nominally equivalent to 1.0 mg/mL of acetazolamide prepared as follows. Transfer a portion of the powder, from NLT 20 Tablets, equivalent to 100 mg acetazolamide into a 100-mL volumetric flask. Add 10 mL of 0.5 N sodium hydroxide, sonicate for 5 min, cool to room temperature, and dilute with water to volume. Filter a portion of this solution, discarding the first 20 mL of the filtrate.

**Sample solution:** Nominally equivalent to 0.1 mg/mL of acetazolamide prepared as follows. Transfer 10.0 mL of *Sample stock solution* and 10 mL of 0.5 N sodium hydroxide to a 100-mL volumetric flask, and dilute with water to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetazolamide ( $C_4H_6N_4O_3S_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = acetazolamide peak response from the *Sample solution*

$r_S$  = acetazolamide peak response from the *Standard solution*

$C_S$  = concentration of USP Acetazolamide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acetazolamide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0%

**PERFORMANCE TESTS**• **DISSOLUTION (711)**

**Medium:** 0.01 N hydrochloric acid; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 60 min

**Standard solution:** USP Acetazolamide RS in *Medium*

**Sample solution:** Dilute with *Medium* if necessary.

**Instrumental conditions**

(See *Spectrophotometry and Light-Scattering* (851).)

**Mode:** UV

**Analytical wavelength:** 265 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of acetazolamide ( $C_4H_6N_4O_3S_2$ ) dissolved:

$$(A_U/A_S) \times C_S \times D \times (V/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$D$  = dilution factor of the *Sample solution*, if needed

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of acetazolamide ( $C_4H_6N_4O_3S_2$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

- **USP REFERENCE STANDARDS (11)**

USP Acetazolamide RS

**Glacial Acetic Acid**

$C_2H_4O_2$

60.05

Acetic acid [64-19-7].

**DEFINITION**

Glacial Acetic Acid contains NLT 99.5% and NMT 100.5%, by weight, of  $C_2H_4O_2$ .

**IDENTIFICATION**

- **IDENTIFICATION TESTS—GENERAL, Acetate (191):** Meets the requirements

**Sample solution** (for lanthanum nitrate test): Glacial Acetic Acid and water (1:100)

**ASSAY**• **PROCEDURE**

**Sample solution:** Measure 2 mL of Glacial Acetic Acid into a glass-stoppered flask, previously tared while containing about 20 mL of water, and weigh again to obtain the weight of the substance under assay.

**Analysis:** Add 20 mL of water, then add phenolphthalein TS. Titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 60.05 mg of  $C_2H_4O_2$ .

**Acceptance criteria:** 99.5%–100.5%

**IMPURITIES****Inorganic Impurities**

- **LIMIT OF NONVOLATILE RESIDUE:** Evaporate 20 mL in a tared dish, and dry at 105° for 1 h: the weight of the residue does not exceed 1.0 mg.



**Delete the following:**

- **HEAVY METALS** (231): NMT 5 ppm  
*Sample solution:* To the residue obtained in the test for *Limit of Nonvolatile Residue* add 8 mL of 0.1 N hydrochloric acid, warm gently until solution is complete, dilute with water to 100 mL, and use 20 mL. (Official 1-Jan-2018)
  - **CHLORIDE AND SULFATE, Chloride** (221)  
*Sample solution:* Dilute 1.0 mL with 20 mL of water.  
*Analysis:* Add 5 drops of silver nitrate TS.  
*Acceptance criteria:* No opalescence is produced.
  - **CHLORIDE AND SULFATE, Sulfate** (221)  
*Sample solution:* Dilute 1.0 mL with 10 mL of water.  
*Analysis:* Add 1 mL of barium chloride TS.  
*Acceptance criteria:* No turbidity is produced.
- Organic Impurities**
- **PROCEDURE: READILY OXIDIZABLE SUBSTANCES**  
*Sample solution:* Dilute 2.0 mL in a glass-stoppered vessel with 10 mL of water.  
*Analysis:* Add 0.10 mL of 0.10 N potassium permanganate.  
*Acceptance criteria:* The pink color is not changed to brown within 2 h.

**SPECIFIC TESTS**

- **CONGEALING TEMPERATURE** (651): NLT 15.6°

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.

**Acetic Acid Irrigation****DEFINITION**

Acetic Acid Irrigation is a sterile solution of Glacial Acetic Acid in Water for Injection. It contains, in each 100 mL, NLT 237.5 mg and NMT 262.5 mg of  $C_2H_4O_2$ .

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Acetate** (191)  
*Sample:* 100 mL of Acetic Acid Irrigation  
*Analysis:* Evaporate the *Sample* to about 10 mL.  
*Acceptance criteria:* The resulting solution meets the requirements.

**ASSAY**• **PROCEDURE**

*Sample:* 50 mL of Acetic Acid Irrigation  
*Analysis:* Pipet the *Sample* into a 150-mL conical flask, add 2 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Each mL of 0.1 N sodium hydroxide is equivalent to 6.005 mg of acetic acid ( $C_2H_4O_2$ ).  
*Acceptance criteria:* 237.5–262.5 mg of  $C_2H_4O_2$  in each 100 mL of Acetic Acid Irrigation

**SPECIFIC TESTS**

- **PH** (791): 2.8–3.4
- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.5 USP Endotoxin Unit/mL.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections* and *Implanted Drug Products* (1), except that the container in which it is packaged may be designed to empty rapidly and may exceed 1000 mL in capacity.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I or Type II glass, and store at controlled room temperature. It may be packaged in suitable plastic containers.

- **USP REFERENCE STANDARDS** (11)  
 USP Endotoxin RS

**Acetic Acid Otic Solution****DEFINITION**

Acetic Acid Otic Solution is a solution of Glacial Acetic Acid in a suitable nonaqueous solvent. It contains NLT 85.0% and NMT 130.0% of the labeled amount of  $C_2H_4O_2$ .

**IDENTIFICATION**

- **A.**  
*Sample solution:* Dilute 5 mL of Acetic Acid Otic Solution with 10 mL of water.  
*Analysis:* Adjust the *Sample solution* with 1 N sodium hydroxide to a pH of 7. Add ferric chloride TS.  
*Acceptance criteria:* A deep red color is produced, and it is destroyed by the addition of hydrochloric acid.
- **B.**  
*Analysis:* Warm it with sulfuric acid and alcohol.  
*Acceptance criteria:* Ethyl acetate, recognizable by its characteristic odor, is evolved.

**ASSAY**• **PROCEDURE**

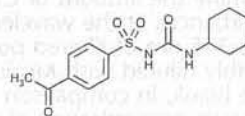
*Sample:* A quantity of Acetic Acid Otic Solution containing 100 mg of glacial acetic acid  
*Analysis:* Transfer the *Sample* to a 250-mL conical flask, and add 5 mL of saturated sodium chloride solution, 40 mL of water, and 3 drops of phenolphthalein TS. Titrate with 0.1 N sodium hydroxide VS to a faint pink endpoint. Each mL of 0.1 N sodium hydroxide is equivalent to 6.005 mg of acetic acid ( $C_2H_4O_2$ ).  
*Acceptance criteria:* 85.0%–130.0%

**SPECIFIC TESTS**

- **PH** (791)  
*Sample solution:* Acetic Acid Otic Solution and water (1:1)  
*Acceptance criteria:* 2.0–4.0

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

**Acetohexamide**

$C_{15}H_{20}N_2O_4S$  324.40  
 Benzenesulfonamide, 4-acetyl-N-[[cyclohexylamino]carbonyl]-  
 1-[(p-Acetylphenyl)sulfonyl]-3-cyclohexylurea [968-81-0].

» Acetohexamide contains not less than 97.0 percent and not more than 101.0 percent of  $C_{15}H_{20}N_2O_4S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—  
 USP Acetohexamide RS



**Identification—****A:** Infrared Absorption (197K).**B:** Ultraviolet Absorption (197U)—*Solution:* 10 µg per mL.*Medium:* 0.01 N sodium hydroxide.

Absorptivities at 247 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 182.5° and 187°.**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.**Residue on ignition** (281): not more than 0.1%.**Selenium** (291): 0.003%, a 200-mg specimen mixed with 200 mg of magnesium oxide being used.**Delete the following:****• Heavy metals, Method II** (231): 0.002%. (Official 1-Jan-2018)**Assay**—Dissolve about 300 mg of Acetohexamide, accurately weighed, in 40 mL of dimethylformamide, add 5 drops of thymol blue TS, and titrate, using a magnetic stirrer, with 0.1 N sodium methoxide VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium methoxide is equivalent to 32.44 mg of C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>S.**Acetohexamide Tablets**» Acetohexamide Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>S.**Packaging and storage**—Preserve in well-closed containers.**USP Reference standards** (11)—

USP Acetohexamide RS

**Identification**—Evaporate on a steam bath to dryness a 20-mL portion of the diluted chloroform solution prepared as directed in the Assay: the residue meets the requirements of Identification test A under Acetohexamide.**Dissolution** (711)—*Medium:* pH 7.6 phosphate buffer (see pH (791)); 900 mL.*Apparatus 1:* 100 rpm.*Time:* 60 minutes.*Procedure*—Determine the amount of C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>S dissolved from UV absorbances at the wavelength of maximum absorbance at about 245 nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, using *Medium* as the blank, in comparison with a Standard solution having a known concentration of USP Acetohexamide RS in the same *Medium*.*Tolerances*—Not less than 75% (Q) of the labeled amount of C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>S is dissolved in 60 minutes.**Uniformity of dosage units** (905): meet the requirements.**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of acetohexamide, to a 100-mL volumetric flask, add 60 mL of 0.1 N sodium hydroxide, and shake for 30 minutes. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Transfer 20.0 mL of the subsequent filtrate to a 125-mL separator, add 2 mL of 3 N hydrochloric acid, and extract with four 40-mL portions of chloroform, filtering each portion through chloroform-washed paper into a 200-mL volumetric flask. Dilute with chloroform to volume, and mix. Transfer20.0 mL of this solution to a suitable beaker, and evaporate on a steam bath to dryness. Transfer the residue, with the aid of 0.1 N sodium hydroxide, to a 100-mL volumetric flask, add 0.1 N sodium hydroxide to volume, and mix. Transfer 10.0 mL of this solution to a third 100-mL volumetric flask, dilute with water to volume, and mix. Concomitantly determine the absorbances of the solution from the Tablets and a Standard solution prepared from USP Acetohexamide RS, in the same medium, at a concentration of about 10 µg per mL, in 1-cm cells, at the wavelength of maximum absorbance at about 247 nm, with a suitable spectrophotometer, using 0.01 N sodium hydroxide as the blank. Calculate the quantity, in mg, of C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>S in the portion of Tablets taken by the formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Acetohexamide RS in the Standard solution; and A<sub>U</sub> and A<sub>S</sub> are the absorbances of the solution from the Tablets and the Standard solution, respectively.**Acetohydroxamic Acid**C<sub>2</sub>H<sub>5</sub>NO<sub>2</sub>

75.07

N-Acetyl hydroxyacetamide;

Acetohydroxamic acid [546-88-3].

**DEFINITION**Acetohydroxamic Acid, dried over phosphorus pentoxide for 16 h, contains NLT 98.0% and NMT 101.0% of acetohydroxamic acid (C<sub>2</sub>H<sub>5</sub>NO<sub>2</sub>).**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197K)• **B.***Sample solution:* 20 mg/mL in water*Analysis:* To 10 mL of the *Sample solution* add 2 drops of potassium permanganate TS.*Acceptance criteria:* The pink color of the permanganate disappears.**ASSAY**• **PROCEDURE***Ferric chloride solution:* 20 mg/mL of ferric chloride in 0.1 N hydrochloric acid*Standard solution:* 500 µg/mL of USP Acetohydroxamic Acid RS in 0.1 N hydrochloric acid*Sample solution:* 500 µg/mL of Acetohydroxamic Acid, previously dried, in 0.1 N hydrochloric acid*Blank:* 0.1 N hydrochloric acid**Analysis***Samples:* *Standard solutions*, *Sample solution*, and *Blank*Transfer 10.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to separate 100-mL volumetric flasks. To each flask add 50 mL of 0.1 N hydrochloric acid and 10.0 mL of *Ferric chloride solution*, and dilute with 0.1 N hydrochloric acid to volume. Without delay, concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 502 nm using the *Blank* to set the instrument.Calculate the percentage of acetohydroxamic acid (C<sub>2</sub>H<sub>5</sub>NO<sub>2</sub>) in the portion of Acetohydroxamic Acid taken:

$$\text{Result} = (A_U / A_S) \times (C_S / C_U) \times 100$$



- $A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of USP Acetohydroxamic Acid RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = concentration of Acetohydroxamic Acid in the *Sample solution* ( $\mu\text{g/mL}$ )

Acceptance criteria: 98.0%–101.0% on the previously dried basis

## IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

## Delete the following:

- **HEAVY METALS, Method I** (231)  
*Sample solution:* Dissolve 1 g in 23 mL of water, and add 2 mL of 1 N acetic acid.  
*Acceptance criteria:* NMT 20 ppm (Official, 1-Jan-2018)
- **LIMIT OF HYDROXYLAMINE**  
*Buffer:* 1.36 g/L of monobasic potassium phosphate in water, adjusted with 1 M potassium hydroxide to a pH of 7.4  
*Solution A:* 1 mg/mL of pyridoxal 5-phosphate monohydrate in *Buffer*, prepared in a low-actinic flask fresh before use  
*Standard stock solution:* 2.0 mg/mL of hydroxylamine hydrochloride in water  
*Standard solutions:* Transfer 5.0, 10.0, and 15.0 mL of the *Standard stock solution* to separate 100-mL volumetric flasks, and dilute with water to volume.  
*Sample solution:* Transfer 1500 mg of Acetohydroxamic Acid, previously dried, to a 100-mL beaker, and dissolve in a sufficient amount of water to cover the electrode of a calibrated pH meter (about 60 mL). While stirring, adjust with 0.05 M potassium hydroxide to a pH of 7.4. Transfer the contents of the beaker, with the aid of small portions of water, to a 100-mL volumetric flask, and dilute with water to volume.  
*Blank:* Water  
*Analysis*  
*Samples:* *Standard solutions*, *Sample solution*, and *Blank*  
 Transfer 2.0 mL of each *Standard solution*, the *Sample solution*, and *Blank* into separate 100-mL volumetric flasks. To each flask add 4.0 mL of *Solution A*. After 8 min, accurately timed, dilute the contents of each flask with *Buffer* to volume.  
 Immediately determine the fluorescence intensities of the solutions from the *Standard solutions* and the *Sample solution* in a fluorometer at an excitation wavelength of 350 nm and an emission wavelength of 450 nm, setting the instrument to zero with the *Blank*. Determine the best-fit straight line from the fluorescence intensities of the three *Standard solutions* versus the hydroxylamine hydrochloride concentrations, in  $\mu\text{g/mL}$ . From the best-fit straight line, determine the concentration, in  $\mu\text{g/mL}$ , of hydroxylamine hydrochloride in the *Sample solution*.  
 Calculate the percentage of hydroxylamine in the portion of Acetohydroxamic Acid taken:

$$\text{Result} = (C_U/C) \times (M_{r1}/M_{r2}) \times 100$$

- $C_U$  = concentration of hydroxylamine hydrochloride in the *Sample solution* (mg/mL)  
 $C$  = concentration of Acetohydroxamic Acid in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of hydroxylamine, 33.03  
 $M_{r2}$  = molecular weight of hydroxylamine hydrochloride, 69.50  
 Acceptance criteria: NMT 0.5%

## SPECIFIC TESTS

- **LOSS ON DRYING** (731)  
*Analysis:* Dry a sample over phosphorus pentoxide for 16 h.

Acceptance criteria: NMT 1.0%

- **COMPLETENESS OF SOLUTION** (641): A 1.0-g portion dissolves in 10 mL of water to yield a clear solution.
- **COLOR OF SOLUTION**  
*Sample solution:* 200 mg/mL in water  
*Blank:* Water  
*Instrumental conditions*  
 (See *Ultraviolet-Visible Spectroscopy* (857).)  
*Mode:* UV-Vis  
*Analytical wavelength:* Between 400 and 750 nm  
*Cell:* 1 cm  
 Acceptance criteria: The absorbance is NMT 0.050.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a cool, dry place.
- **USP REFERENCE STANDARDS** (11)  
 USP Acetohydroxamic Acid RS

## Acetohydroxamic Acid Tablets

### DEFINITION

Acetohydroxamic Acid Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acetohydroxamic acid ( $\text{C}_2\text{H}_5\text{NO}_2$ ).

### IDENTIFICATION

- **A.** Tablets produce a purple color when mixed with an acidic solution of ferric chloride.

### ASSAY

- **PROCEDURE**  
*Ferric chloride solution:* 20 mg/mL of ferric chloride in 0.1 N hydrochloric acid  
*Standard solution:* 500  $\mu\text{g/mL}$  of USP Acetohydroxamic Acid RS in 0.1 N hydrochloric acid  
*Sample solution:* Weigh, and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of acetohydroxamic acid, to a 1000-mL volumetric flask, add about 500 mL of 0.1 N hydrochloric acid, and shake for 1 min. Dilute with 0.1 N hydrochloric acid to volume, and mix. Filter, discarding the first 40 mL of the filtrate. Use the clear filtrate.  
*Blank:* 0.1 N hydrochloric acid  
*Analysis*  
*Samples:* *Standard solutions*, *Sample solution*, and *Blank*  
 Transfer 10.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to separate 100-mL volumetric flasks. To each flask add 50 mL of 0.1 N hydrochloric acid and 10.0 mL of *Ferric chloride solution*, and dilute with 0.1 N hydrochloric acid to volume. Without delay, concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 502 nm, using the *Blank* to set the instrument.  
 Calculate the percentage of labeled amount of acetohydroxamic acid ( $\text{C}_2\text{H}_5\text{NO}_2$ ) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- $A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of USP Acetohydroxamic Acid RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of acetohydroxamic acid in the *Sample solution* ( $\mu\text{g/mL}$ )



Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION, Procedure for a Pooled Sample (711)

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Analysis: Calculate the percentage of the labeled amount of acetohydroxamic acid ( $C_2H_5NO_2$ ) dissolved, using the procedure in the Assay, using a filtered portion of the solution under test as *Sample solution* in comparison with a *Standard solution* having a known concentration of USP Acetohydroxamic Acid RS in Medium.

Tolerances: NLT 85% (Q) of the labeled amount of acetohydroxamic acid ( $C_2H_5NO_2$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

## IMPURITIES

### • LIMIT OF HYDROXYLAMINE

Buffer: 1.36 g/L of monobasic potassium phosphate in water, adjusted with 1 M potassium hydroxide to a pH of 7.4

Solution A: 1 mg/mL of pyridoxal 5-phosphate monohydrate in Buffer, prepared in a low-actinic flask fresh before use

Standard stock solution: 2.0 mg/mL of hydroxylamine hydrochloride in water

Standard solutions: Transfer 5.0, 10.0, and 15.0 mL of the Standard stock solution to separate 100-mL volumetric flasks, and dilute with water to volume.

Sample solution: Weigh, and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to about 1500 mg of acetohydroxamic acid to a 50-mL stoppered centrifuge tube. Add 30.0 mL of water, shake for about 2 min, and centrifuge. Pipet 15.0 mL of the clear solution into a 50-mL beaker, add just enough water to cover the electrode of a calibrated pH meter, and while stirring, adjust with 0.5 M potassium hydroxide to a pH of 7.4. Quantitatively transfer the contents of the beaker with the aid of small portions of water to a 50-mL volumetric flask, dilute with water to volume, and mix.

Blank: Water

### Analysis

Samples: Standard solutions, Sample solution, and Blank. Transfer 2.0 mL of each Standard solution, the Sample solution, and Blank into separate 100-mL volumetric flasks. To each flask add 4.0 mL of Solution A. After 8 min, accurately timed, dilute the contents of each flask with Buffer to volume.

Immediately determine the fluorescence intensities of the solutions from the Standard solutions and the Sample solution in a fluorometer at an excitation wavelength of 350 nm and an emission wavelength of 450 nm, setting the instrument to zero with the Blank. Determine the best-fit straight line from the fluorescence intensities of the three Standard solutions versus the hydroxylamine hydrochloride concentrations, in  $\mu\text{g/mL}$ . From the best-fit straight line, determine the concentration, in  $\mu\text{g/mL}$ , of hydroxylamine hydrochloride in the Sample solution.

Calculate the percentage of hydroxylamine in the portion of Tablets taken:

$$\text{Result} = (C_U/C) \times (M_{r1}/M_{r2}) \times 100$$

$C_U$  = concentration of hydroxylamine hydrochloride in the Sample solution ( $\mu\text{g/mL}$ )

$C$  = nominal concentration of acetohydroxamic acid in the Sample solution ( $\mu\text{g/mL}$ )

$M_{r1}$  = molecular weight of hydroxylamine, 33.03

$M_{r2}$  = molecular weight of hydroxylamine hydrochloride, 69.50

Acceptance criteria: NMT 0.5%

## ADDITIONAL REQUIREMENTS

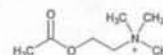
### • PACKAGING AND STORAGE:

Preserve in tight containers.

### • USP REFERENCE STANDARDS (11)

USP Acetylcholine Chloride RS

## Acetylcholine Chloride



$C_7H_{16}ClNO_2$  181.66

Ethanaminium, 2-(acetoxy)-N,N,N-trimethyl-, chloride;

Choline acetate (ester) chloride [60-31-1].

## DEFINITION

Acetylcholine Chloride contains NLT 98.0% and NMT 102.0% of acetylcholine chloride ( $C_7H_{16}ClNO_2$ ), calculated on the dried basis.

## IDENTIFICATION

### • A. INFRARED ABSORPTION (197K)

### • B.

Sample solution: 100 mg/mL in water

Analysis: To 5 mL of Sample solution add 5 mL of silver nitrate TS.

Acceptance criteria: A white, curdy precipitate, which is soluble in ammonium hydroxide but insoluble in nitric acid, is formed.

## ASSAY

### • PROCEDURE

Sample: 400 mg of Acetylcholine Chloride

Analysis: Dissolve in 15 mL of water in a glass-stoppered conical flask, add 40.0 mL of 0.1 N sodium hydroxide VS, and heat on a steam bath for 30 min. Insert the stopper, allow to cool, add phenolphthalein TS, and titrate the excess alkali with 0.1 N sulfuric acid VS. Perform a blank determination (see *Titrimetry* (541), *Residual Titrations*). Each mL of 0.1 N sodium hydroxide is equivalent to 18.17 mg of  $C_7H_{16}ClNO_2$ .

Acceptance criteria: 98.0%–102.0% on the dried basis

## OTHER COMPONENTS

### • CONTENT OF CHLORIDE

Sample: 280 mg of Acetylcholine Chloride

Analysis: Dissolve the Sample in 140 mL of water, and add 1 mL of dichlorofluorescein TS. Titrate with 0.1 N silver nitrate VS until the silver chloride flocculates and the mixture acquires a faint pink color. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl.

Acceptance criteria: 19.3%–19.8% of Cl on the dried basis

## IMPURITIES

### • RESIDUE ON IGNITION (281):

NMT 0.2%

## SPECIFIC TESTS

### • MELTING RANGE OR TEMPERATURE, Class I (741):

149°–152°

### • ACIDITY

Sample: 100 mg of Acetylcholine Chloride

Analysis: Dissolve the Sample in 10 mL of recently boiled water, and add at once 1 drop of bromothymol blue TS.

Acceptance criteria: NMT 0.50 mL of 0.010 N sodium hydroxide is required to produce a color change.



• **LOSS ON DRYING** (731)

Analysis: Dry a sample at 105° for 3 h.

Acceptance criteria: NMT 1.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in a tight container, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Acetylcholine Chloride RS

## Acetylcholine Chloride for Ophthalmic Solution

**DEFINITION**

Acetylcholine Chloride for Ophthalmic Solution is a sterile mixture of Acetylcholine Chloride with Mannitol or other suitable diluent, prepared by freeze-drying. Each container contains NLT 90.0% and NMT 115.0% of the labeled amount of acetylcholine chloride ( $C_7H_{16}ClNO_2$ ).

**IDENTIFICATION**

• **A.**

**Standard solution:** 10 mg/mL of USP Acetylcholine Chloride RS

**Sample solution:** 10 mg/mL of acetylcholine chloride

**Chromatographic system**

(See Chromatography (621), Thin-Layer Chromatography.)

**Adsorbent:** 0.25-mm layer of aluminum oxide

**Application volume:** 2  $\mu$ L

**Developing solvent system:** Mix butyl alcohol, glacial acetic acid, and water (40:10:50). Allow the layers to separate completely. Use the upper layer.

**Spray reagent A:** Freshly prepared solution of 5 mg/mL of cobaltous chloride prepared as follows. Dissolve the required amount of cobaltous chloride in 50% of the final volume of water, and dilute with 50% alcohol. [NOTE—This solution is freshly prepared.]

**Spray reagent B:** Freshly prepared potassium ferrocyanide solution prepared as follows. Dissolve 1.0 g of potassium ferrocyanide in 100 mL of water, and dilute with 50 mL of alcohol.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Develop the chromatogram, without delay, in a vapor-saturated chamber containing the *Developing solvent system*. Allow the solvent front to move about 10 cm beyond the initial spotting line. Dry the plate with a current of warm air. Immediately spray the plate with *Spray reagent A*. Dry the plate as before, and immediately spray the plate with *Spray reagent B*. Dry the plate with a current of warm air.

**Acceptance criteria:** The  $R_f$  value and color of the principal spot from the *Sample solution* correspond to those from the *Standard solution*.

• **B.**

**Sample solution:** Nominally 10 mg/mL of acetylcholine chloride

**Analysis:** To 2 mL of *Sample solution* add 1 drop of nitric acid and 1 mL of silver nitrate TS.

**Acceptance criteria:** A curdy, white precipitate, soluble in an excess of 6 N ammonium hydroxide, is formed.

**ASSAY**

• **PROCEDURE**

**Mobile phase:** Add 1.03 g of sodium 1-heptanesulfonate to a mixture of 900 mL of water and 10 mL of methanol. Adjust with ammonium hydroxide or glacial acetic acid to a pH of 4.0. Add 50 mL of acetonitrile. Dilute with water to 1 L. [NOTE—A slight variation of the amount of acetonitrile may be required to improve resolution or adjust retention time.]

**Standard solution:** A quantity of USP Acetylcholine Chloride RS in *Mobile phase*, to obtain a solution having a known concentration equal to that of the acetylcholine chloride in the *Sample solution*

**Sample solution:** Transfer the contents of 1 container of Acetylcholine Chloride for Ophthalmic Solution to a 10-mL volumetric flask with the aid of *Mobile phase*, and dilute with *Mobile phase* to volume.

**System suitability solution:** 0.2% each of acetylcholine chloride and choline chloride

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 50  $\mu$ L

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between acetylcholine chloride and choline chloride, *System suitability solution*

**Relative standard deviation:** NMT 3.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acetylcholine chloride ( $C_7H_{16}ClNO_2$ ) in the container taken:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Acetylcholine Chloride RS in the *Standard solution* (mg/mL)

$V$  = volume of the *Sample solution*, 10 mL

$L$  = label claim (mg/vial)

**Acceptance criteria:** 90.0%–115.0%

**PERFORMANCE TESTS**

• **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

**SPECIFIC TESTS**

• **STERILITY TESTS** (71): Meets the requirements

• **ACIDITY**

**Analysis:** Dissolve an amount of Acetylcholine Chloride for Ophthalmic Solution equivalent to 100 mg of acetylcholine chloride in 10 mL of recently boiled water. Add at once 1 drop of bromothymol blue TS.

**Acceptance criteria:** NMT 0.50 mL of 0.010 N sodium hydroxide is required to produce a color change.

• **WATER DETERMINATION, Method I** (921)

**Analysis:** Perform the titration in the original container, observing precautions against contact with water or moist atmosphere. Adjust the concentration of the reagent so that the titration volume approaches but does not exceed the capacity of the container. Titrate to an amber color that persists for 15 s after mixing.

**Acceptance criteria:** NMT 1.0%

• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections* and *Implanted Drug Products* (1), *Specific Tests*, *Completeness* and *clarity* of solutions.

**ADDITIONAL REQUIREMENTS**

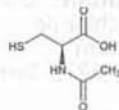
**Change to read:**

• **PACKAGING AND STORAGE:** Preserve in tight containers as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for constitution* (CN 1-May-2017), and store at controlled room temperature.



- **USP REFERENCE STANDARDS (11)**  
USP Acetylcholine Chloride RS

## Acetylcysteine



$C_5H_9NO_3S$   
L-Cysteine, N-acetyl-;  
N-Acetyl-L-cysteine [616-91-1].

163.19

### DEFINITION

Acetylcysteine contains NLT 98.0% and NMT 102.0% of  $C_5H_9NO_3S$ , calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

### ASSAY

- **PROCEDURE**

**Mobile phase:** 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.

**Sodium metabisulfite solution:** 0.5 mg/mL of sodium metabisulfite in water, freshly prepared

**Internal standard solution:** 5 mg/mL of USP L-Phenylalanine RS in Sodium metabisulfite solution

**Standard stock solution:** 10 mg/mL of USP Acetylcysteine RS in Sodium metabisulfite solution

**Standard solution:** 0.5 mg/mL of USP Acetylcysteine RS and 0.25 mg/mL of USP L-Phenylalanine RS in Sodium metabisulfite solution from Standard stock solution and Internal standard solution

**Sample stock solution:** 10 mg/mL of Acetylcysteine in Sodium metabisulfite solution

**Sample solution:** 0.5 mg/mL of Acetylcysteine and 0.25 mg/mL of USP L-Phenylalanine RS in Sodium metabisulfite solution from Sample stock solution and Internal standard solution

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 5  $\mu$ L

#### System suitability

**Sample:** Standard solution

[NOTE—The relative retention times for acetylcysteine and L-phenylalanine are about 0.5 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 6 between acetylcysteine and L-phenylalanine

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** Standard solution and Sample solution  
Calculate the percentage of acetylcysteine ( $C_5H_9NO_3S$ ) in the portion of Acetylcysteine taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of acetylcysteine to L-phenylalanine from the Sample solution

$R_S$  = peak response ratio of acetylcysteine to L-phenylalanine from the Standard solution

$C_S$  = concentration of USP Acetylcysteine RS in the Standard solution (mg/mL)

$C_U$  = concentration of acetylcysteine in the Sample solution (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.5%

### Delete the following:

- **HEAVY METALS, Method II (231)**

[CAUTION—Exercise care because explosion may occur.]

**Analysis:** In a dropwise manner, wet the sample with 2 mL of nitric acid, and proceed as directed for the Test preparation.

Acceptance criteria: NMT 10 ppm (Official 1-Jan-2018)

### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation (781S)**

**Buffer:** Mix 29.5 mL of 1 N sodium hydroxide, 50 mL of 1 M monobasic potassium phosphate, and sufficient water to make 100 mL. Adjust to a pH of  $7.0 \pm 0.1$  by adding more of either solution, as necessary.

**Sample solution:** In a 25-mL volumetric flask, mix 1.25 g with 1 mL of edetate disodium solution (1 in 100), add 7.5 mL of sodium hydroxide solution (1 in 25), and mix to dissolve. Dilute with Buffer to volume.

Acceptance criteria:  $+21^\circ$  to  $+27^\circ$

- **pH (791):** 2.0–2.8 in a solution (1 in 100)

- **LOSS ON DRYING (731):** Dry a sample at a pressure of about 50 mm of mercury at  $70^\circ$  for 4 h; it loses NMT 1.0% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

- **USP REFERENCE STANDARDS (11)**

USP Acetylcysteine RS

USP L-Phenylalanine RS

## Acetylcysteine Solution

### DEFINITION

Acetylcysteine Solution is a sterile solution of Acetylcysteine in water, prepared with the aid of Sodium Hydroxide. It contains NLT 90.0% and NMT 110.0% of the labeled amount of acetylcysteine ( $C_5H_9NO_3S$ ).

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

**Sample solution:** Place 10 mL in a suitable beaker, and adjust to a pH of 2 (pH indicator paper), using 3 N hydrochloric acid. Add up to 2 g of finely powdered sodium chloride, in two portions of 200 mg each initially and then in smaller portions of 25 mg, stirring after each addition until the sodium chloride dissolves and a precipitate is formed. The precipitate appears as a very fine powder, and the solution turns cloudy. If no precipitate forms, add an additional drop of 3 N hydrochloric acid, and stir until the precipitate forms. Allow to stand at room temperature for 15 min, and collect the residue by suction filtration. Use the acetylcysteine so obtained after being dried at a pressure of 50 mm of mercury at  $70^\circ$  for 4 h.

Acceptance criteria: Meets the requirements

### ASSAY

- **PROCEDURE**

**Solution A:** 0.5 mg/mL of sodium metabisulfite solution in water, freshly prepared

**Solution B:** 0.5 mg/mL of sodium bisulfite solution

**Mobile phase:** 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.



**Internal standard solution:** 5 mg/mL of USP L-Phenylalanine RS in *Solution A*

**Standard stock solution:** 10 mg/mL of USP Acetylcysteine RS in *Solution A*

**Standard solution:** 0.5 mg/mL of USP Acetylcysteine RS and 0.25 mg/mL of USP L-Phenylalanine RS in *Solution A* from *Standard stock solution* and *Internal standard solution*

**Sample stock solution:** Equivalent to 10 mg/mL of acetylcysteine from the volume of *Solution in Solution B*

**Sample solution:** 0.5 mg/mL of acetylcysteine and 0.25 mg/mL of USP L-Phenylalanine RS in *Solution A* from *Standard stock solution* and *Internal standard solution*

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 5 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 6 between acetylcysteine and L-phenylalanine

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for acetylcysteine and L-phenylalanine are about 0.5 and 1.0, respectively.]

Calculate the percentage of the labeled amount of acetylcysteine ( $C_5H_9NO_3S$ ) in the portion of *Solution* taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of acetylcysteine to L-phenylalanine from the *Sample solution*

$R_S$  = peak response ratio of acetylcysteine to L-phenylalanine from the *Standard solution*

$C_S$  = concentration of USP Acetylcysteine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acetylcysteine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

• **pH** <791>: 6.0–7.5

• **STERILITY TESTS** <71>: Meets the requirements

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-unit or multiple-unit tight containers that effectively exclude oxygen, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** <11>

USP Acetylcysteine RS

USP L-Phenylalanine RS

### Acetylcysteine and Isoproterenol Hydrochloride Inhalation Solution

» Acetylcysteine and Isoproterenol Hydrochloride Inhalation Solution is a sterile solution of Acetylcysteine and Isoproterenol Hydrochloride in water. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of acetylcysteine ( $C_5H_9NO_3S$ ), and not less than 90.0 percent and not more than

115.0 percent of the labeled amount of isoproterenol hydrochloride ( $C_{11}H_{17}NO_3 \cdot HCl$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, tightly closed with a glass or polyethylene closure, and store at controlled room temperature.

**Labeling**—The label indicates that the Inhalation Solution is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

#### USP Reference standards <11>—

USP Acetylcysteine RS

USP Isoproterenol Hydrochloride RS

USP L-Phenylalanine RS

**Color and clarity**—Using the Inhalation Solution as the *Test solution*, proceed as directed for *Color and clarity* under *Isoproterenol Inhalation Solution*.

#### Identification—

**A:** Place 2 mL in a 10-mL beaker, and adjust with 3 N hydrochloric acid to a pH of about 3 (pH indicator paper). Add 500 mg to 1 g of finely powdered sodium chloride, in two portions of about 200 mg each initially, and then in smaller portions (about 25 mg), stirring after each addition, until a precipitate is formed. Allow to stand at room temperature for 15 minutes, and collect the residue by suction filtration: the acetylcysteine so obtained, after being dried as directed in the test for *Loss on drying* under *Acetylcysteine*, responds to the *Identification* test under *Acetylcysteine*.

**B:** *Ferro-Citrate Solution* and *Buffer Solution*—Prepare as directed under *Epinephrine Assay* <391>.

**Procedure**—Place a volume of Inhalation Solution, equivalent to about 0.26 mg of isoproterenol hydrochloride, in a test tube with 3 mL of 0.1 M mercuric chloride, and mix. Add 100 µL of *Ferro-Citrate Solution* and 1.0 mL of *Buffer Solution*, and mix: the presence of isoproterenol hydrochloride is confirmed by the development of a purple color.

**Sterility Tests** <71>: meets the requirements.

**pH** <791>: between 6.0 and 7.0.

#### Assay for acetylcysteine—

*Mobile phase*, *Internal standard solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the Assay under *Acetylcysteine*.

**Assay preparation**—Pipet a volume of Inhalation Solution, equivalent to about 1000 mg of acetylcysteine, into a 100-mL volumetric flask, dilute with sodium metabisulfite solution (1 in 2000) to volume, and mix. Pipet 10 mL of this solution and 10 mL of *Internal standard solution* into a 200-mL volumetric flask, dilute with sodium metabisulfite solution (1 in 2000) to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Acetylcysteine*. Calculate the quantity, in mg, of  $C_5H_9NO_3S$  in each mL of the Inhalation Solution taken by the formula:

$$2000(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Acetylcysteine RS in the *Standard preparation*; V is the volume, in mL, of Inhalation Solution taken; and  $R_U$  and  $R_S$  are the ratios of the peak response of acetylcysteine to that of DL-phenylalanine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for isoproterenol hydrochloride—

*Mobile phase*—Dissolve 13.6 g of monobasic potassium phosphate in 1000 mL of water, and pass through a membrane filter having a 0.45-µm porosity. Add 20.0 mL of methanol, mix, and degas.

*Internal standard solution*—Place about 150 mg of acetaminophen in a 500-mL volumetric flask, add 5 mL of glacial acetic acid, dilute with water to volume, and mix.



**Standard preparation**—Dissolve an accurately weighed quantity of USP Isoproterenol Hydrochloride RS in 0.05 M sodium metabisulfite to obtain a solution having a known concentration of 0.15 mg per mL. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with 0.2 M acetic acid to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Inhalation Solution, equivalent to about 1.5 mg of isoproterenol hydrochloride, and 10 mL of *Internal standard solution* to a 25-mL volumetric flask, add dilute glacial acetic acid (1 in 100) to volume, and mix.

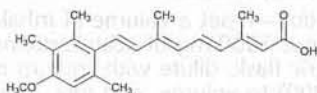
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm × 40-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for isoproterenol hydrochloride and 1.0 for acetaminophen; the resolution,  $R_s$ , between isoproterenol hydrochloride and acetaminophen is not less than 6; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of isoproterenol hydrochloride ( $C_{11}H_{17}NO_3 \cdot HCl$ ) in each mL of the Inhalation Solution taken by the formula:

$$(25C/V)(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Isoproterenol Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Inhalation Solution taken; and  $R_U$  and  $R_S$  are the ratios of the peak responses of isoproterenol hydrochloride to those of acetaminophen obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Acitretin



$C_{21}H_{26}O_3$  326.43  
2,4,6,8-Nonatetraenoic acid, 9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-, (*all-E*); (*all-E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid [55079-83-9].

### DEFINITION

Acitretin contains NLT 98.0% and NMT 102.0% of  $C_{21}H_{26}O_3$ , calculated on the dried basis.

**[CAUTION]**—Acitretin is a teratogen. Great care should be taken when handling to avoid inhalation of dust or contact with skin.]

[NOTE—Use low-actinic glassware and perform all tests under yellow and subdued light.]

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

[NOTE—Store the solutions at 4° before injection.]

**Mobile phase:** Alcohol, glacial acetic acid, and water (92:0.3:8)

**System suitability stock solution:** 0.01 mg/mL each of USP Acitretin RS and USP Tretinoin RS in alcohol.

[NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]

**System suitability solution:** 0.25  $\mu$ g/mL each of USP Acitretin RS and USP Tretinoin RS in alcohol, from *System suitability stock solution*

**Standard solution:** 0.1 mg/mL of USP Acitretin RS in alcohol. [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol. The final concentration of tetrahydrofuran in the preparation will be 2%.]

**Sample stock solution:** 0.25 mg/mL of Acitretin in tetrahydrofuran and alcohol (1:19). [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]

**Sample solution:** 0.1 mg/mL of Acitretin in alcohol, from *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 360 nm

**Column:** 4-mm × 25-cm; packing L1

**Flow rate:** 0.6 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for tretinoin and acitretin are 0.84 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between tretinoin and acitretin, *System suitability solution*

**Relative standard deviation:** NMT 1.0% of acitretin, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{21}H_{26}O_3$  in the portion of Acitretin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Acitretin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Acitretin in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

#### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.1%

#### Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1: Jan-2018)

#### Organic Impurities

[NOTE—Store the solutions at 4° before injection.]

#### • PROCEDURE

**Mobile phase and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.8  $\mu$ g/mL each of USP Acitretin RS, USP Acitretin Related Compound A RS, and USP Acitretin Related Compound B RS in alcohol. [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]

**Sample solution:** 0.25 mg/mL of Acitretin in tetrahydrofuran and alcohol (1:19). [NOTE—Dissolve in tetrahydrofuran before diluting with alcohol.]



**System suitability**(See *Chromatography* (621), *System Suitability*.)**Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 1.5 between acitretin related compound A and acitretin; NLT 1.5 between acitretin related compound B and acitretin**Relative standard deviation:** NMT 10.0% for acitretin related compound A and NMT 10.0% for acitretin related compound B**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acitretin related compound A and acitretin related compound B in the portion of Acitretin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 $r_u$  = peak response from the relevant impurity from the *Sample solution* $r_s$  = peak response from the relevant impurity from the *Standard solution* $C_s$  = concentration of USP Acitretin Related Compound A RS or USP Acitretin Related Compound B RS in the *Standard solution* ( $\mu\text{g/mL}$ ) $C_u$  = concentration of Acitretin in the *Sample solution* ( $\mu\text{g/mL}$ )

Calculate the percentage of impurities other than acitretin related compounds A and B in the portion of Acitretin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 $r_u$  = peak response of each individual unspecified impurity from the *Sample solution* $r_s$  = peak response of USP Acitretin RS in the *Standard solution* $C_s$  = concentration of USP Acitretin RS in the *Standard solution* ( $\mu\text{g/mL}$ ) $C_u$  = concentration of Acitretin in the *Sample solution* ( $\mu\text{g/mL}$ )**Acceptance criteria****Individual impurities:** See *Impurity Table 1*.**Total impurities:** NMT 1.0%**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acitretin related compound A	0.78	0.3
Acitretin	1.0	—
Acitretin related compound B	1.61	0.3
Any unspecified impurity	—	0.1
Total unspecified impurities	—	0.4

**SPECIFIC TESTS**

- Loss on Drying** (731): Dry a sample in a vacuum at a pressure not exceeding 19 mm of mercury at 100° for 4 h: it loses NMT 0.2% of its weight.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.
- USP REFERENCE STANDARDS** (11)
  - USP Acitretin RS
  - USP Acitretin Related Compound A RS (2Z,4E,6E,8E)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid.
  - $\text{C}_{21}\text{H}_{26}\text{O}_3$  326.43

USP Acitretin Related Compound B RS

Ethyl (all-E)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoate.

 $\text{C}_{23}\text{H}_{30}\text{O}_3$  354.48

USP Tretinoin RS

**Acitretin Capsules****DEFINITION**Acitretin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of acitretin ( $\text{C}_{21}\text{H}_{26}\text{O}_3$ ).**[CAUTION]**—Acitretin is a teratogen. Great care should be taken when handling to avoid inhalation of dust or contact with skin.]**[NOTE]**—Use low-actinic glassware and perform all tests under yellow and subdued light. Make all injections within 1 h of *Sample solution* preparation.]**IDENTIFICATION**

- THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Standard solution:** 10 mg/mL of USP Acitretin RS in tetrahydrofuran**Sample solution:** Equivalent to 20 mg of acitretin from Capsules. Grind to a fine powder, then triturate for 30 s with 2 mL of tetrahydrofuran. Transfer the suspension to a 12-mL conical centrifuge tube, and centrifuge to obtain a clear supernatant.**Application volume:** 10  $\mu\text{L}$ **Developing solvent system:** Chloroform and methanol (4:1)**Analysis****Samples:** *Standard solution* and *Sample solution*

Proceed as directed in the chapter, and then air-dry.

Spray the plate with a saturated solution of antimony trichloride in chloroform (250 mg/mL) followed by concentrated sulfuric acid, and then locate the spots.

**ASSAY**

- PROCEDURE**

**Diluent:** Methanol and tetrahydrofuran (13:10)**Mobile phase:** Methanol, alcohol, glacial acetic acid, and water (74: 5: 0.5: 21)**Standard solution:** 0.1 mg/mL of USP Acitretin RS in a mixture of *Diluent* and water (23:2). Dissolve USP Acitretin RS in *Diluent* equivalent to 80% of the final volume, sonicate for 5 min, add water equivalent to 8% of the final volume, and dilute with *Diluent* to volume.**System suitability solution:** Transfer 2 mL of the *Standard solution* to a clear 4-mL glass vial. After sealing the vial with a teflon-lined silicone septum and cap, place the vial on its side in a light chamber, expose it to 400 foot-candles of fluorescent light for 5 min, and then completely wrap the vial with aluminum foil.**[NOTE]**—Exposure to the fluorescent light allows for the formation of two degradation products: acitretin related compound A and 6Z-isomer. See *Table 1* for the relative retention times.]**Sample solution:** 0.1 mg/mL of acitretin in a mixture of *Diluent* and water (23:2). Open NLT 20 Capsules, composite the Capsule fill, and mix well. Transfer the Capsule fill to a volumetric flask, add water equivalent to 8% of the final volume to wet the sample, and sonicate for 5 min. Dilute with *Diluent* to volume, and sonicate for 5 min. Cool to room temperature, pass the suspension through a suitable filter of 0.5- $\mu\text{m}$  pore size, and use the clear filtrate. **[NOTE]**—Inject the *Sample solution* within 1 h of preparation.]**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 365 nm  
 Column: 4.6-mm × 15-cm; 5-μm packing L1  
 Flow rate: 1 mL/min  
 Injection size: 25 μL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 3.0 between acitretin related compound A and acitretin; NLT 1.8 between the 6Z-isomer and acitretin, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of acitretin ( $C_{21}H_{26}O_3$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Acitretin RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION (711)****Test 1**

**Medium:** 3% sodium lauryl sulfate in deaerated water, pH 9.6–10.0; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

Determine the amount of acitretin ( $C_{21}H_{26}O_3$ ) dissolved using the following method.

**Standard solution:** Transfer about 14 mg of USP Acitretin RS to a 500-mL volumetric flask. Dissolve in 50 mL of alcohol, and dilute with *Medium* to volume.

**For Capsules labeled to contain 10 mg:** Transfer 20 mL of this solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.

**Sample solution:** Use portions of the solution under test passed through a suitable filter of 0.45-μm pore size.

**Capsule shell solution:** Dissolve 6 clean empty-shell Capsules in 900 mL of *Medium*.

**Analytical wavelength:** 347 nm

**Cell length:** 2 mm

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Capsule shell solution*

Calculate the amount of acitretin ( $C_{21}H_{26}O_3$ ) dissolved:

$$\text{Result} = [(A_U - A_{CS})/A_S] \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*  
 $A_{CS}$  = Capsule shell correction, calculated as shown below  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of the appropriate *Standard solution* (mg/mL)  
 $L$  = Capsule label claim (mg)  
 $V$  = volume of *Medium*, 900 mL

The Capsule shell correction,  $A_{CS}$ , is calculated:

$$A_{CS} = A_{CSS}/N$$

$A_{CSS}$  = absorbance of the *Capsule shell solution*  
 $N$  = number of Capsule shells used to prepare the *Capsule shell solution*

**Tolerances:** NLT 85% (Q) of the labeled amount of acitretin ( $C_{21}H_{26}O_3$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Tier 1**

**Medium:** 3% sodium lauryl sulfate in deaerated water, pH 9.6–10.0 (adjusted with 1 N sodium hydroxide); 900 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

Determine the amount of acitretin ( $C_{21}H_{26}O_3$ ) dissolved using the following method.

**Tier 2**

**Medium A:** Prepare a solution containing pancreatin with NMT 1750 USP units of protease activity/L in deaerated water, pH 8.0 (adjusted with 1% sodium hydroxide); 450 mL, use immediately.

**Medium B:** 6% sodium lauryl sulfate in deaerated water, pH 10.5 (adjusted with 1% sodium hydroxide); 450 mL

**Apparatus 1:** 100 rpm

**Time:** 10 min *Medium A*; 20 min *Medium A* with the addition of *Medium B*

**Mobile phase:** Methanol, water, and glacial acetic acid (750:250:1)

**Standard stock solution:** 280 μg/mL of USP Acitretin RS in absolute alcohol. Use sonication to dissolve.

**Standard solution:** 20 μg/mL of USP Acitretin RS in *Medium* under *Tier 1*, from *Standard stock solution*

**Sample solution:** Pass a portion of the solution under test through a suitable glass filter with 1-μm pore size, discard first few mL, and use the filtrate for analysis.

**Dissolution procedure:** Perform the test using the conditions under *Tier 1*. In the presence of cross-linking repeat the test with new Capsules using the conditions under *Tier 2* as follows. After 10 min, stop the dissolution bath and timer (do not lift the baskets), and add 450 mL of *Medium B* pre-equilibrated at  $37 \pm 0.5^\circ$ . Restart the timer and bath, and after 5 min check the pH of the medium and adjust to a range of 9.6–10.0 with 1% sodium hydroxide. Continue dissolution for an additional 15 min.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** 360 nm

**Columns**

**Guard:** 4-mm × 1-cm; 5-μm packing L1

**Analytical:** 4.6-mm × 5-cm; 5-μm packing L1

**Temperatures**

**Column:**  $35^\circ$

**Autosampler:**  $40^\circ$

**Flow rate:** 2.0 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acitretin ( $C_{21}H_{26}O_3$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Acitretin RS in the *Standard solution* (mg/mL)  
 $L$  = label claim (mg/Capsule)  
 $V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 85% (Q) of the labeled amount of acitretin ( $C_{21}H_{26}O_3$ ) is dissolved.

**• UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements



**IMPURITIES****• ORGANIC IMPURITIES: LIMIT OF DEGRADATION PRODUCTS**

Diluent, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

**Analysis**

Sample: Sample solution

Calculate the percentage of each degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each individual impurity

$r_T$  = sum of the responses of all the peaks

Acceptance criteria: See Table 1.

Table 1

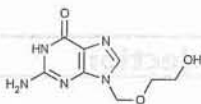
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Acicretin related compound A(2Z-isomer) <sup>a</sup>	0.84	0.5
Acicretin	1.0	—
6Z-Isomer <sup>b</sup>	1.09	—
Any unspecified impurity	—	0.4
Total unspecified impurities	—	0.8

<sup>a</sup> [(2Z,4E,6E,8E)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid] (C<sub>21</sub>H<sub>26</sub>O<sub>3</sub> 326.43).

<sup>b</sup> (2E,4E,6Z,8E)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **LABELING:** When more than one Dissolution test is given, the labeling states the test used only if Test 1 is not used.
- **USP REFERENCE STANDARDS (11)**  
USP Acicretin RS

**Acyclovir**

C<sub>8</sub>H<sub>11</sub>N<sub>5</sub>O<sub>3</sub> 225.20

6H-Purin-6-one, 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-.

9-[(2-Hydroxyethoxy)methyl]guanine [59277-89-3].

» Acyclovir contains not less than 98.0 percent and not more than 101.0 percent of C<sub>8</sub>H<sub>11</sub>N<sub>5</sub>O<sub>3</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers. Store at room temperature. Protect from light and moisture.

**USP Reference standards (11)**—

USP Acyclovir RS

**Identification**—

A: Infrared Absorption (197K).

B: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay and limit for guanine.

**Water Determination, Method I (921):** not more than 6.0%.

**Ordinary impurities (466)**—

Test solution: dimethyl sulfoxide.

Standard solution: dimethyl sulfoxide.

Eluant: a mixture of chloroform, methanol, and ammonium hydroxide (80:20:2).

Visualization: 1.

Application volume: 5  $\mu$ L.

Limit: 1%.

**Assay and limit for guanine**—

**Mobile phase**—Prepare a filtered and degassed solution of glacial acetic acid in water (1 in 1000). Make adjustments if necessary (see System Suitability under Chromatography (621)).

**System suitability solution 1**—Dissolve accurately weighed quantities of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having known concentrations of about 0.1 mg of each per mL.

**System suitability solution 2**—Dissolve an accurately weighed quantity of guanine in 0.1 N sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.7  $\mu$ g per mL.

**Guanine standard preparation**—Transfer about 8.75 mg of guanine, accurately weighed, to a 500-mL volumetric flask. Dissolve in 50 mL of 0.1 N sodium hydroxide, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.01 N sodium hydroxide to volume, and mix to obtain a solution having a concentration of about 0.7  $\mu$ g per mL.

**Standard preparation**—Dissolve about 25 mg of USP Acyclovir RS, accurately weighed, in 5 mL of 0.1 N sodium hydroxide in a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.01 N sodium hydroxide to volume, and mix to obtain a solution having a known concentration of about 0.1 mg of USP Acyclovir RS per mL.

**Assay preparation**—Dissolve about 100 mg of Acyclovir, accurately weighed, in 20 mL of 0.1 N sodium hydroxide in a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with 0.01 N sodium hydroxide to volume, and mix.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 3 mL per minute. Chromatograph System suitability solution 1, and record the peak responses as directed for Procedure: the resolution,  $R$ , between acyclovir and guanine is not less than 2.0; the tailing factor for the analyte peak is not more than 2; and the relative standard deviation for replicate injections for the acyclovir peak is not more than 2.0%. Chromatograph System suitability solution 2, and record the peak responses as directed for Procedure: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the Standard preparation, the Guanine standard preparation, and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in  $\mu$ g, of guanine in the portion of Acyclovir taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of guanine in the Guanine standard preparation; and  $r_U$  and  $r_S$  are the peak responses due to guanine in the Assay preparation and the Guanine standard preparation, respectively: not more than



0.7% of guanine is found. Calculate the quantity, in mg, of  $C_8H_{11}N_5O_3$  in the portion of Acyclovir taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Acyclovir RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses due to acyclovir in the *Assay preparation* and the *Standard preparation*, respectively.

## Acyclovir Capsules

### DEFINITION

Acyclovir Capsules contain NLT 93.0% and NMT 107.0% of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Mobile phase:** 0.02 M acetic acid

**System suitability solution A:** 0.1 mg/mL each of USP Acyclovir RS and guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**System suitability solution B:** 2.0 µg/mL of guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**Standard solution:** 0.1 mg/mL of USP Acyclovir RS. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**Sample solution:** Nominally 0.1 mg/mL of acyclovir prepared as follows. Transfer the contents of Capsules equivalent to 10 mg of acyclovir (NLT 10 Capsules) to a 100-mL volumetric flask. Dissolve in 10 mL of 0.1 N sodium hydroxide, dilute to volume with water, and filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.2-mm × 25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution A* and *System suitability solution B*

[NOTE—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in *System suitability solution A*.]

#### Suitability requirements

**Resolution:** NLT 2.0 between guanine and acyclovir, *System suitability solution A*

**Relative standard deviation:** NMT 2.0% for the acyclovir peak, *System suitability solution A*

**Relative standard deviation:** NMT 2.0%, *System suitability solution B*

**Analysis:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the *Sample solution*

$r_S$  = peak response of the *Standard solution*

$C_S$  = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Detector:** UV 254 nm

**Standard solution:** USP Acyclovir RS in *Medium*

**Sample solutions:** Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

**Analysis:** Determine the amount of acyclovir ( $C_8H_{11}N_5O_3$ ) dissolved from UV absorption at the wavelength of maximum absorption on filtered portions of the solution under test.

**Tolerances:** NLT 75% (Q) of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Content Uniformity*

### IMPURITIES

#### • PROCEDURE

**Mobile phase, System suitability solution A, System suitability solution B, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis:** *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses for all of the peaks

#### Acceptance criteria

**Guanine:** NMT 2.0%

**Any individual impurity:** NMT 0.5%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25°. Protect from light and moisture.

#### • USP REFERENCE STANDARDS (11)

USP Acyclovir RS

## Acyclovir for Injection

### DEFINITION

Acyclovir for Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Mobile phase:** 0.02 M acetic acid

**System suitability solution A:** 0.1 mg/mL each of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide

**System suitability solution B:** 2.0 µg/mL of guanine in 0.1 N sodium hydroxide

**Standard solution:** 0.1 mg/mL of USP Acyclovir RS in 0.1 N sodium hydroxide

**Sample solution:** Nominally 0.1 mg/mL of acyclovir prepared as follows. Constitute 1 vial of Acyclovir for Injection with water. Transfer an amount, equivalent to 10 mg of acyclovir, to a 100-mL volumetric flask, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 254 nm

Column: 4.2-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

#### System suitability

Samples: System suitability solution A and System suitability solution B

[NOTE—The relative retention times for guanine and acyclovir are 0.6 and 1.0, respectively, in System suitability solution A.]

#### Suitability requirements

Resolution: NLT 2.0 between guanine and acyclovir, System suitability solution A

Relative standard deviation: NMT 2.0% for the acyclovir peak, System suitability solution A

Relative standard deviation: NMT 2.0%, System suitability solution B

#### Analysis

Calculate the percentage of acyclovir ( $C_8H_{11}N_5O_3$ ) in the portion of Acyclovir for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the Sample solution

$r_S$  = peak response of the Standard solution

$C_S$  = concentration of USP Acyclovir RS in the Standard solution (mg/mL)

$C_U$  = concentration of the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### IMPURITIES

##### PROCEDURE

Solution A: 0.17 M acetic acid and methanol (125:8)

Solution B: Methanol

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
45	65	35
46	100	0
56	100	0

System suitability solution: 0.5 µg/mL each of purine and USP Acyclovir RS in Solution A

Acyclovir standard solution: 5 µg/mL of USP Acyclovir RS in Solution A

Guanine solution: 0.05 mg/mL of guanine prepared as follows. Dissolve 25 mg of guanine in 50 mL of 0.1 N sodium hydroxide in a 500-mL volumetric flask, and bring the solution to volume with water.

Standard solution A: 0.5 µg/mL of Acyclovir standard solution in Solution A

Standard solution B: 5 µg/mL of Guanine solution in Solution A

Sample solution: Equivalent to 0.5 mg/mL of acyclovir from a mixture of NLT 10 reconstituted vials of Acyclovir for Injection in Solution A

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

#### System suitability

Samples: System suitability solution, Standard solution A, and Standard solution B

[NOTE—Typical retention times for guanine and acyclovir of Standard solution A and Standard solution B are 5.8 and 14 min, respectively.]

#### Suitability requirements

Resolution: NLT 2.0 between purine and acyclovir, System suitability solution

Relative standard deviation: NMT 1% for the acyclovir and the guanine peaks, Standard solution A and Standard solution B

#### Analysis 1

Calculate the percentage of guanine in the Acyclovir for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for guanine, if present, in the Sample solution

$r_S$  = peak response of guanine in the Standard solution

$C_S$  = concentration of guanine in the Standard solution (mg/mL)

$C_U$  = nominal concentration of acyclovir in the Sample solution (mg/mL)

Acceptance criteria 1: NMT 1.0% guanine

#### Analysis 2

Calculate the percentage of each other impurity in the portion of Acyclovir for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for each impurity

$r_S$  = peak response of acyclovir in the Standard solution

$C_S$  = concentration of USP Acyclovir RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of acyclovir in the Sample solution (mg/mL)

Acceptance criteria 2: NMT 0.15% for any peak having a relative retention time of about 0.7 compared to the acyclovir peak; NMT 0.5% for any other individual impurity; and NMT 1.0% for the total of all other impurities

#### SPECIFIC TESTS

- **PH (791):** 11.0–12.5, 50 mg/mL of acyclovir
- **WATER DETERMINATION, Method I (921):** NMT 5.5%
- **STERILITY TESTS (71):** Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.174 USP Endotoxin Unit/mg of acyclovir
- **OTHER REQUIREMENTS:** Meets the requirements for labeling in Labeling (7), Labels and Labeling for Injectable Products

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25°. Protect from light.
- **USP REFERENCE STANDARDS (11)**  
USP Acyclovir RS  
USP Endotoxin RS

## Acyclovir Ointment

#### DEFINITION

Acyclovir Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ), in a suitable ointment base.

#### IDENTIFICATION

- **A.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.



**ASSAY**• **PROCEDURE**

Mobile phase: 0.02 M acetic acid

System suitability solution A: 0.1 mg/mL each of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide

System suitability solution B: 2.0 µg/mL of guanine in 0.1 N sodium hydroxide

Standard solution: 0.1 mg/mL of USP Acyclovir RS in 0.1 N sodium hydroxide

Sample solution: Nominally 0.1 mg/mL of acyclovir prepared as follows. Transfer an amount of Ointment, equivalent to 10 mg of acyclovir, to a 100-mL volumetric flask. Dissolve in and dilute with 0.1 N sodium hydroxide to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 3 mL/min

Injection volume: 20 µL

**System suitability**

Samples: System suitability solution A and System suitability solution B

[NOTE—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in System suitability solution A.]

**Suitability requirements**

Resolution: NLT 2.0 between guanine and acyclovir, System suitability solution A

Relative standard deviation: NMT 2.0% for the acyclovir peak, System suitability solution A; NMT 2.0%, System suitability solution B

**Analysis**

Samples: Standard solution and Sample solution  
Calculate the percentage of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Acyclovir RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of acyclovir in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**

- **MINIMUM FILL (755):** Meets the requirements

**IMPURITIES**• **LIMIT OF GUANINE**

Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 2.0 µg/mL of guanine in 0.1 M sodium hydroxide

**Analysis**

Samples: Standard solution and Sample solution  
Calculate the percentage of guanine in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of guanine from the Sample solution

$r_S$  = peak response of guanine from the Standard solution

$C_S$  = concentration of guanine in the Standard solution (mg/mL)

$C_U$  = nominal concentration of acyclovir in the Sample solution (mg/mL)

Acceptance criteria: NMT 2.0%

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25° in a dry place.
- **USP REFERENCE STANDARDS (11)**  
USP Acyclovir RS

**Acyclovir Oral Suspension****DEFINITION**

Acyclovir Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

Mobile phase: 0.02 M acetic acid

System suitability solution A: 0.1 mg/mL each of USP Acyclovir RS and guanine in 0.1 N sodium hydroxide

System suitability solution B: 2.0 µg/mL of guanine in 0.1 N sodium hydroxide

Standard solution: 0.1 mg/mL of USP Acyclovir RS in 0.1 N sodium hydroxide

Sample stock solution: Nominally 1 mg/mL of acyclovir prepared as follows. Transfer an amount of well-shaken Oral Suspension equivalent to 200 mg of acyclovir to a 200-mL volumetric flask. Add 100 mL of 0.1 N sodium hydroxide, shake by mechanical means for 15 min, and sonicate, if necessary, to dissolve the Oral Suspension completely. Dilute with 0.1 N sodium hydroxide to volume.

Sample solution: Transfer 10.0 mL of the Sample stock solution to a 100-mL volumetric flask, and dilute with water to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 3 mL/min

Injection volume: 20 µL

**System suitability**

Samples: System suitability solution A and System suitability solution B

[NOTE—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in System suitability solution A.]

**Suitability requirements**

Resolution: NLT 2.0 between guanine and acyclovir, System suitability solution A

Relative standard deviation: NMT 2.0% for replicate injections for the acyclovir peak, System suitability solution A

Relative standard deviation: NMT 2.0% for replicate injections, System suitability solution B



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**IMPURITIES**• **LIMIT OF GUANINE**

**Mobile phase, System suitability solution A, System suitability solution B, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** 2.0 µg/mL of guanine in 0.1 M sodium hydroxide

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of guanine in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response for guanine from the *Sample solution*  
 $r_S$  = peak response for guanine from the *Standard solution*  
 $C_S$  = concentration of guanine in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.0%

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): Its total count does not exceed  $10^1$  cfu/mL, and it meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.
- **PH** (791): 4.5–7.0

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements for Oral Suspension packaged in single-unit containers
- **DELIVERABLE VOLUME** (698): Meets the requirements for Oral Suspension packaged in multiple-unit containers

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25°. Protect from light.
- **USP REFERENCE STANDARDS** (11)  
USP Acyclovir RS

**Acyclovir Tablets****DEFINITION**

Acyclovir Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** 0.02 M acetic acid

**System suitability solution A:** 0.1 mg/mL each of USP Acyclovir RS and guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**System suitability solution B:** 2.0 µg/mL of guanine. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**Standard solution:** 0.1 mg/mL of USP Acyclovir RS. Dissolve in 0.1 N sodium hydroxide, and dilute with water.

**Sample solution:** Nominally 0.1 mg/mL of acyclovir prepared as follows. Transfer an amount of finely powdered Tablets equivalent to 10 mg of acyclovir (NLT 10 Tablets) to a 100-mL volumetric flask. Dissolve in 10 mL of 0.1 N sodium hydroxide, dilute with water to volume, and filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *System suitability solution A* and *System suitability solution B*

[NOTE—The relative retention times for guanine and acyclovir are about 0.6 and 1.0, respectively, in *System suitability solution A*.]

**Suitability requirements**

**Resolution:** NLT 2.0 between guanine and acyclovir, *System suitability solution A*

**Relative standard deviation:** NMT 2.0% for the acyclovir peak, *System suitability solution A*; NMT 2.0%, *System suitability solution B*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Acyclovir RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of acyclovir in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION** (711)

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Instrumental conditions**

**Mode:** UV

**Wavelength:** 254 nm

**Standard solution:** USP Acyclovir RS in *Medium*

**Sample solutions:** Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

**Analysis:** Determine the amount of acyclovir ( $C_8H_{11}N_5O_3$ ) dissolved from UV absorption at the wavelength of maximum absorbance on filtered portions of the solution under test.

**Tolerances:** NLT 80% (Q) of the labeled amount of acyclovir ( $C_8H_{11}N_5O_3$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements for *Weight Variation*



**IMPURITIES****• PROCEDURE**

Mobile phase, System suitability solution A, System suitability solution B, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

**Analysis**

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses for all of the peaks

**Acceptance criteria**

Guanine: NMT 2.0%

Any other impurity: NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 25°. Protect from light and moisture.

**• USP REFERENCE STANDARDS (11)**

USP Acyclovir RS

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

**System suitability**

Sample: *Standard solution*

**Suitability requirements**

Relative standard deviation: NMT 1.0%

**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of adapalene (C<sub>28</sub>H<sub>28</sub>O<sub>3</sub>) in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

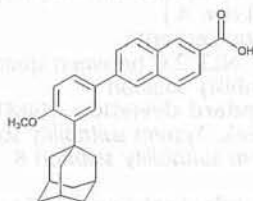
$C_S$  = concentration of USP Adapalene RS in the *Standard solution* (μg/mL)

$C_U$  = concentration of Adapalene in the *Sample solution* (μg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

**IMPURITIES**

- RESIDUE ON IGNITION (281):** NMT 0.20%

**Adapalene**

C<sub>28</sub>H<sub>28</sub>O<sub>3</sub> 412.52

2-Naphthalenecarboxylic acid, 6-(4-methoxy-3-tricyclo[3.3.1.1<sup>3,7</sup>]dec-1-ylphenyl)-;

6-[3-(1-Adamantyl)-4-methoxyphenyl]-2-naphthoic acid. [106685-40-9].

**DEFINITION**

Adapalene contains NLT 98.0% and NMT 102.0% of adapalene (C<sub>28</sub>H<sub>28</sub>O<sub>3</sub>), calculated on the dried basis.

**IDENTIFICATION****• A. INFRARED ABSORPTION (197K)**

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****• PROCEDURE**

**Mobile phase:** Acetonitrile, tetrahydrofuran, trifluoroacetic acid, and water (21: 16: 0.01: 13)

**Standard stock solution:** 0.2 mg/mL of USP Adapalene RS in *Mobile phase*. Dissolve USP Adapalene RS in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.

**Standard solution:** 40 μg/mL of USP Adapalene RS in *Mobile phase* from the *Standard stock solution*

**Sample stock solution:** 0.2 mg/mL of Adapalene in *Mobile phase*. Dissolve Adapalene in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.

**Sample solution:** 40 μg/mL of Adapalene in *Mobile phase* from the *Sample stock solution*

**Delete the following:**

- HEAVY METALS, Method II (231):** NMT 20 μg/g (Official 1-

Jan-2018)

[NOTE—On the basis of the synthetic route, perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*.]

**• ORGANIC IMPURITIES, PROCEDURE 1**

*Procedure 1* is recommended if adapalene related compounds A and B may be present.

**Mobile phase:** Proceed as directed in the Assay.

**Standard stock solution:** 0.2 mg/mL of USP Adapalene RS, 0.3 mg/mL of USP Adapalene Related Compound A RS, and 0.2 mg/mL of USP Adapalene Related Compound B RS in *Mobile phase*. Dissolve USP Adapalene RS, USP Adapalene Related Compound A RS, and USP Adapalene Related Compound B RS in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.

**Standard solution:** 0.2 μg/mL of USP Adapalene RS, 0.3 μg/mL of USP Adapalene Related Compound A RS, and 0.2 μg/mL of USP Adapalene Related Compound B RS in *Mobile phase* from the *Standard stock solution*

**Sample solution:** 0.2 mg/mL of Adapalene in *Mobile phase*. Dissolve Adapalene in a minimal amount of tetrahydrofuran (about 1%–5% of the final volume), using sonication as needed, and dilute with *Mobile phase* to volume.

**Chromatographic system:** Proceed as directed in the Assay, except use a run time of NLT two times the retention time of adapalene peak for *Standard solution* and NLT six times the retention time of adapalene peak for *Sample solution*.

**System suitability**

Sample: *Standard solution*

**Suitability requirements**

Relative standard deviation: NMT 3.0% for the adapalene peak

Column efficiency: NLT 3000 theoretical plates for the adapalene peak



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of adapalene related compounds A and B in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak area of each impurity from the *Sample solution*  
 $r_S$  = peak area of corresponding adapalene related compound A or adapalene related compound B from the *Standard solution*  
 $C_S$  = concentration of corresponding USP Adapalene Related Compound A RS or USP Adapalene Related Compound B RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Adapalene in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak area of each unspecified impurity from the *Sample solution*  
 $r_S$  = peak area of adapalene from the *Standard solution*  
 $C_S$  = concentration of USP Adapalene RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Adapalene in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 1. Disregard any impurity peaks less than 0.05%.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Adapalene related compound A <sup>a</sup>	0.52	0.10
Adapalene	1.0	—
Adapalene related compound B <sup>b</sup>	1.57	0.10
Any individual unspecified impurity	—	0.10
Total impurities	—	0.50

<sup>a</sup> Methyl 6-bromo-2-naphthoate.

<sup>b</sup> Methyl 6-[3-(1-Adamantyl)-4-methoxyphenyl]-2-naphthoate.

**• ORGANIC IMPURITIES, PROCEDURE 2**

*Procedure 2* is recommended if adapalene related compounds E, C, and D may be present.

**Solution A:** Glacial acetic acid and water (0.1:100)

**Solution B:** Acetonitrile and tetrahydrofuran (65:35)

**Mobile phase:** See Table 2.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	50	50
2.5	50	50
40	28	72
42	28	72
42.1	50	50
50	50	50

**Diluent:** Acetonitrile, tetrahydrofuran, and water (37:20:43)

**Standard stock solution:** 0.2 mg/mL of USP Adapalene RS in tetrahydrofuran

**Standard solution:** 2.0 µg/mL of USP Adapalene RS in *Diluent* from the *Standard stock solution*

**System suitability solution:** 0.2 mg/mL of USP Adapalene RS and 1.2 µg/mL each of USP Adapalene Related Compound C RS, USP Adapalene Related Compound D RS, and USP Adapalene Related Compound E RS prepared by dissolving the standards in tetrahydrofuran equivalent to 50% of the final volume, and diluting with *Diluent* to volume

**Sample solution:** 2.0 mg/mL of Adapalene prepared by dissolving in tetrahydrofuran equivalent to 50% of the final volume, and diluting with *Diluent* to volume

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L11 with 7.5% carbon loading

**Column temperature:** 30°

**Flow rate:** 1.2 mL/min

**Injection volume:** 25 µL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 4.5 between the adapalene and adapalene related compound C peaks

**Signal-to-noise ratio:** NLT 10 for the adapalene related compound C peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- $r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of adapalene from the *Standard solution*  
 $C_S$  = concentration of adapalene in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Adapalene in the *Sample solution* (mg/mL)  
 $F$  = relative response factor for each individual impurity (see Table 3)  
**Acceptance criteria:** See Table 3. Disregard any impurity peaks less than 0.05%.

**Table 3**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Adapalene related compound E <sup>a</sup>	0.3	1.4	0.3
Hydroxyadapalene <sup>b</sup>	0.5	0.91	0.1
Adapalene related compound C <sup>c</sup>	0.9	0.14	0.1
Adapalene	1.0	—	—
Adapalene related compound D <sup>d</sup>	1.9	0.71	0.2
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

<sup>a</sup> 2,2'-Binaphthyl-6,6'-dicarboxylic acid.

<sup>b</sup> 6-[3-(3-Hydroxyadamant-1-yl)-4-methoxyphenyl]-2-naphthoic acid.

<sup>c</sup> 2-(Adamant-1-yl)methoxybenzene.

<sup>d</sup> 4,4'-Dimethoxy-3,3'-di(adamant-1-yl)biphenyl.



• **RESIDUAL SOLVENT: LIMIT OF TRIETHYLAMINE**

[NOTE—This test should be performed if triethylamine is used in the manufacturing process.]

**Diluent:** Dimethyl sulfoxide

**Standard solution:** 4.0 µg/mL of USP Triethylamine RS in *Diluent*. Transfer 4.0 mL of this solution to a 20-mL headspace vial, and add 1.0 mL of 1 N NaOH solution.

**Sample solution:** 50 mg/mL of Adapalene in *Diluent*. Transfer 4.0 mL of this solution to a 20-mL headspace vial, and add 1.0 mL of 1 N NaOH solution.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 30-m × 0.53-mm; 3.0-µm coating of G27

**Temperatures**

**Injection port:** 250°

**Detector:** 300°

**Column:** See *Table 4*.

**Table 4**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	5
40	40	240	5

**Headspace operating parameters**

[NOTE—Headspace operating parameters can be modified in order to optimize the performance.]

**Equilibration temperature:** 95°

**Equilibration time:** 15 min

**Transfer line temperature:** 125°

**Pressurization time:** 3 min

**Carrier gas:** Nitrogen

**Flow rate:** 4.8 mL/min

**Injection volume:** 1 mL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 15%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the content, in ppm, of triethylamine in the portion of Adapalene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 10^6$$

$r_U$  = peak response of triethylamine from the *Sample solution*

$r_S$  = peak response of triethylamine from the *Standard solution*

$C_S$  = concentration of triethylamine in the *Standard solution* (mg/mL)

$C_U$  = concentration of Adapalene in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 80 ppm

**SPECIFIC TESTS**

• **LOSS ON DRYING (731)**

**Analysis:** Dry a sample at 105° for 4 h.

**Acceptance criteria:** NMT 0.6%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.

• **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies.

• **USP REFERENCE STANDARDS (11)**

USP Adapalene RS

USP Adapalene Related Compound A RS

Methyl 6-bromo-2-naphthoate.

$C_{12}H_9BrO_2$  265.10

USP Adapalene Related Compound B RS

Methyl 6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoate.

$C_{29}H_{30}O_3$  426.55

USP Adapalene Related Compound C RS

2-(Adamant-1-yl)methoxybenzene.

$C_{17}H_{22}O$  242.36

USP Adapalene Related Compound D RS

4,4'-Dimethoxy-3,3'-di(adamant-1-yl)biphenyl.

$C_{34}H_{42}O_2$  482.70

USP Adapalene Related Compound E RS

2,2'-Binaphthyl-6,6'-dicarboxylic acid.

$C_{22}H_{14}O_4$  342.34

USP Triethylamine RS

Triethylamine.

$C_6H_{15}N$  101.19

## Adapalene Gel

**DEFINITION**

Adapalene Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of adapalene ( $C_{28}H_{28}O_3$ ).

**IDENTIFICATION**

• **A. ULTRAVIOLET ABSORPTION (197U)**

**Diluent:** Use *Mobile phase* in the *Assay*.

**Sample stock solution:** Use *Sample stock solution* in the *Assay*.

**Sample solution:** Nominally equivalent to 0.4 µg/mL of adapalene, prepared as follows. Dilute 2.0 mL of *Sample stock solution* with *Diluent* to 100.0 mL. Pass a portion through a Teflon filter of 0.45-µm pore size and use the filtrate.

**Acceptance criteria:** Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

• **PROCEDURE**

**Mobile phase:** Acetonitrile, tetrahydrofuran, trifluoroacetic acid, and water (43: 36: 0.02: 21)

**Standard stock solution:** 0.25 mg/mL of USP Adapalene RS, prepared as follows. Transfer USP Adapalene RS to a suitable volumetric flask, add tetrahydrofuran equivalent to 1% of the final volume, and sonicate to dissolve. Dilute with *Mobile phase* to volume.

**Standard solution:** 20 µg/mL of USP Adapalene RS in *Mobile phase*, from *Standard stock solution*

**Sample stock solution:** Nominally equivalent to 20 µg/mL of adapalene, prepared as follows. Transfer 2.0 g of Gel to a 100-mL volumetric flask, add 25 mL of tetrahydrofuran, and sonicate to dissolve. Add 25 mL of acetonitrile and sonicate for 20 min. Cool to room temperature and dilute with *Diluent* to volume.

**Sample solution:** Pass a portion of *Sample stock solution* through a Teflon filter of 0.45-µm pore size and use the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 235 nm  
 Column: 4.6-mm × 25-cm; 5-μm packing L1  
 Flow rate: 1 mL/min  
 Injection volume: 20 μL

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of adapalene ( $C_{28}H_{28}O_3$ ) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Adapalene RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of adapalene in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

### IMPURITIES

#### • ORGANIC IMPURITIES

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Solution A: Use *Mobile phase* in the Assay.

Solution B: Buffer and Solution A (50:50)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	0	100
4	0	100
30	55	45
65	55	45
68	0	100
80	0	100

Diluent: Acetonitrile and tetrahydrofuran (3:2)

System suitability stock solution: 0.5 mg/mL of USP Adapalene RS, prepared as follows. Transfer USP Adapalene RS to a suitable volumetric flask, add tetrahydrofuran equivalent to 40% of the final volume, and sonicate to dissolve. Dilute with acetonitrile to volume.

System suitability solution: 0.2 mg/mL of USP Adapalene RS in Diluent, from System suitability stock solution

Standard solution: 1.0 μg/mL of USP Adapalene RS in Diluent, from System suitability solution

Sample solution: Nominally equivalent to 0.2 mg/mL of adapalene, prepared as follows. Transfer 5.0 g of Gel to a 25-mL volumetric flask. Add 10 mL of tetrahydrofuran and sonicate to disperse for 10 min. Add 10 mL of acetonitrile and sonicate for 10 min. Cool to room temperature and dilute with acetonitrile to volume. Pass a portion through a Teflon filter of 0.45-μm pore size and use the filtrate.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 μL

#### System suitability

Samples: *System suitability solution* and *Standard solution*

#### Suitability requirements

Tailing factor: NMT 2.0, System suitability solution

Relative standard deviation: NMT 5.0%, Standard solution

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of each impurity from the *Sample solution*

$r_S$  = peak area of adapalene from the *Standard solution*

$C_S$  = concentration of USP Adapalene RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of adapalene in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard any peak less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Adapalene related compound A <sup>a,b</sup>	0.5	—
Adapalene	1.0	—
Adapalene related compound B <sup>b,c</sup>	1.3	—
Any unspecified impurity	—	0.2
Total impurities	—	1.0

<sup>a</sup> Methyl 6-bromo-2-naphthoate.

<sup>b</sup> This process impurity is controlled in the drug substance monograph. It is included in the table for identification only and it is not to be reported in the total impurities.

<sup>c</sup> Methyl 6-[3-(adamant-1-yl)-4-methoxyphenyl]-2-naphthoate.

### SPECIFIC TESTS

• **pH (791):** 4.0–6.0

• **MINIMUM FILL (755):** Meets the requirements

• **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count is NMT  $10^2$  cfu/g. The total yeasts and molds count is NMT  $10^1$  cfu/g. It meets the requirements of the tests for the absence of *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* species.

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature and protect from freezing.

• **USP REFERENCE STANDARDS (11)**  
 USP Adapalene RS



## Adenine



$C_5H_5N_5$  135.13  
1*H*-Purin-6-amine;  
1,6-Dihydro-6-iminopurine [73-24-5].

### DEFINITION

Adenine contains NLT 98.0% and NMT 102.0% of adenine ( $C_5H_5N_5$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer solution:** Dissolve 6.90 g of monobasic ammonium phosphate in about 800 mL of water. Adjust with ammonium hydroxide to a pH of 6.2, and dilute with water to 1 L.

**Mobile phase:** See Table 1.

Table 1

Time (min)	Buffer Solution (%)	Acetonitrile (%)	Water (%)
0	5	5	90
20	5	5	90
20.1	10	10	80
30	10	10	80
30.1	5	5	90
40	5	5	90

**System suitability solution:** 50 µg/mL each of USP Adenine RS and 7-methyladenine in water

**Standard solution:** 0.1 mg/mL of USP Adenine RS in water. If necessary, sonicate the solution at 30° until the substance is completely dissolved.

**Sample solution:** 0.1 mg/mL of Adenine in water. If necessary, sonicate the solution at 30° until the substance is completely dissolved.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 260 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L85

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for 7-methyladenine and adenine are 0.88 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between the 7-methyladenine and adenine peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of adenine ( $C_5H_5N_5$ ) in the portion of Adenine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Adenine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Adenine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

### Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 µg/g • (Official 1-

Jan-2018)

### • RELATED COMPOUNDS

**Buffer solution, Mobile phase, System suitability solution, Standard solution, and System suitability:** Proceed as directed in the *Assay*.

**Sample solution:** Dissolve 25 mg of Adenine in approximately 15 mL of boiling water. Cool, quantitatively transfer to a 25-mL volumetric flask, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L85

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 µL

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Adenine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_T$  = sum of all the peak responses from the *Sample solution*

#### Acceptance criteria

**Individual impurity:** NMT 0.1%

**Total impurities:** NMT 2.0%

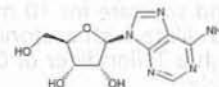
### SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample at 110° for 4 h: it loses NMT 1.0% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP Adenine RS

## Adenosine



$C_{10}H_{13}N_5O_4$  267.24

6-Amino-9-β-D-ribofuranosyl-9*H*-purine;  
9-β-D-Ribofuranosyladenine [58-61-7].

### DEFINITION

Adenosine contains NLT 98.0% and NMT 102.0% of adenosine ( $C_{10}H_{13}N_5O_4$ ), calculated on the dried basis.



**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197M)
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 6.8 g/L of potassium hydrogen sulfate and 3.4 g/L of tetrabutylammonium hydrogen sulfate in a solution prepared as follows. Transfer suitable quantities of potassium hydrogen sulfate and tetrabutyl ammonium hydrogen sulfate to an appropriate volumetric flask, and dissolve in 90% of the flask volume of water. Adjust with 2 N potassium hydroxide to a pH of 6.5, and dilute with water to volume.

**Mobile phase:** Buffer and water (60:40)

**System suitability solution:** 4 µg/mL each of USP Adenine RS and inosine in *Mobile phase*

**Standard solution:** 0.2 mg/mL of USP Adenosine RS in *Mobile phase*

**Sample solution:** 0.2 mg/mL of Adenosine in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**Run time:** NLT 1.5 times the retention time of the adenosine peak

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between adenine and inosine, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 0.7%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of adenosine (C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>) in the portion of Adenosine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Adenosine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Adenosine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**Delete the following:**

- **HEAVY METALS**, *Method II* (231): NMT 10 ppm • (Official 1-Jan-2018)

• **ORGANIC IMPURITIES**

**Buffer, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.001 mg/mL of USP Adenosine RS in *Mobile phase*

**Sample solution:** 1 mg/mL of Adenosine in *Mobile phase*

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between adenine and inosine, *System suitability solution*

**Relative standard deviation:** NMT 5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Adenosine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Adenosine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Adenosine in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*. Disregard peaks that are less than 0.05% of the adenosine peak.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Uridine <sup>a</sup>	0.29	0.73	0.10
Adenine	0.34	1.6	0.2
Inosine <sup>b</sup>	0.42	0.73	0.1
Guanosine <sup>c</sup>	0.51	0.86	0.10
Adenosine	1.0	—	—
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

<sup>a</sup> 1-β-D-Ribofuranosylpyrimidine-2,4(1H,3H)-dione.

<sup>b</sup> 9-β-D-Ribofuranosylpurine-6(1H)-one.

<sup>c</sup> 2-Amino-9-β-D-ribofuranosylpurine-6(1H)-one.

**SPECIFIC TESTS**

- **OPTICAL ROTATION**, *Specific Rotation* (781S): −68° to −72°

**Test solution:** 20 mg/mL in sodium hydroxide solution (1 in 20), determined on a sample previously dried at 105° for 2 h

- **LOSS ON DRYING** (731)

**Analysis:** Dry a sample at 105° for 2 h.

**Acceptance criteria:** NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

- **USP REFERENCE STANDARDS** (11)

USP Adenine RS

USP Adenosine RS

**Adenosine Injection****DEFINITION**

Adenosine Injection is a sterile solution of Adenosine in Water for Injection. It may contain Sodium Chloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of adenosine (C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>).

**IDENTIFICATION**

- The retention time of the adenosine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.



**ASSAY****• PROCEDURE**

**Mobile phase:** Dissolve 2.0 g of monobasic potassium phosphate in 800 mL of water. Add 5 mL of 1.0 M tetrabutylammonium dihydrogen phosphate, dilute with water to 980 mL, and mix. Add 20 mL of acetonitrile.

**System suitability solution:** 0.03 mg/mL each of USP Adenosine RS and inosine dissolved in warm water (50° to 55°), and diluted with water

**Standard solution:** 0.03 mg/mL of USP Adenosine RS dissolved in warm water (50° to 55°), and diluted with water to volume. Before addition of the warm water, if sodium chloride is present in the injection, add 0.01 mL of a solution of sodium chloride (0.9 in 100) per mL of the anticipated final volume of the *Standard solution*.

**Sample solution:** Nominally 0.03 mg/mL of adenosine, from a suitable volume of injection in water

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2.5 mL/min

**Injection volume:** 10 µL

**Run time:** 2.5 times the retention time of adenosine

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times of inosine and adenosine are 0.43 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 6.0 between adenosine and inosine, *System suitability solution*

**Tailing factor:** NMT 2.0 for the adenosine peak, *System suitability solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of adenosine ( $C_{10}H_{13}N_5O_4$ ) in the portion of injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Adenosine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of adenosine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**IMPURITIES****• ORGANIC IMPURITIES**

**Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Sample solution:** Nominally 0.3 mg/mL of adenosine from a volume of injection, in water

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the volume of injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses of all of the peaks

**Acceptance criteria**

**Any individual impurity:** NMT 1.0%

**Total impurities:** NMT 1.5%

**SPECIFIC TESTS**

**• pH (791):** 4.5–7.5

**• PARTICULATE MATTER IN INJECTIONS (788):** It meets the requirements for small-volume injections.

**• BACTERIAL ENDOTOXINS TEST (85):** When the product is used for rapid intravenous injection, it contains NMT 11.62 USP Endotoxin Units/mg of adenosine. When the product is used for continuous peripheral intravenous infusion, it contains NMT 5.95 USP Endotoxin Units/mg of adenosine.

**• OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* (1).

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in tight, single-dose containers, preferably of Type I glass, and store at controlled room temperature.

**• USP REFERENCE STANDARDS (11)**

USP Adenosine RS

USP Endotoxin RS

**Medical Air****DEFINITION**

Medical Air is a natural or synthetic mixture of gases consisting largely of nitrogen and oxygen. It contains NLT 19.5% and NMT 23.5%, by volume, of oxygen ( $O_2$ ).

**IDENTIFICATION**

- **A.** The paramagnetic signal exhibited by the *Sample gas* in the *Assay* confirms the presence of oxygen.
- **B.** The *Sample gas* in the *Assay* meets the assay *Acceptance criteria*.

**ASSAY****• PROCEDURE**

The certified standards called for in the following test are listed in *Reagents, Indicators, and Solutions*.

**Zero gas:** Nitrogen certified standard

**Span gas:** 21% Oxygen certified standard. [NOTE—See *Reagents, Indicators, and Solutions*.]

**Sample gas:** Medical Air

**Mode:** Paramagnetic oxygen measurement (see *Medical Gases Assay* (415))

**Analysis:** Determine the concentration of oxygen in percentage by volume of Medical Air using a suitable paramagnetic analyzer.

**Acceptance criteria:** 19.5%–23.5% of oxygen by volume

**IMPURITIES**

See *Impurities Testing in Medical Gases Assay* (413). The detector tubes called for in the following tests are listed in *Reagents, Indicators, and Solutions*.

If the label indicates that Medical Air is a synthetic mixture of oxygen and nitrogen, and where oxygen complies to *Oxygen USP* and Nitrogen complies to *Nitrogen NF*, then the *Impurities* tests are not required.

**• LIMIT OF CARBON DIOXIDE**

**Sample:** Detector tube manufacturer's recommended volume  $\pm 5\%$  of Medical Air

**Analysis:** Pass the *Sample* through a carbon dioxide detector tube at the rate specified for the tube by the detector tube manufacturer.

**Acceptance criteria:** NMT 500 ppm

**• LIMIT OF CARBON MONOXIDE**

**Sample:** Detector tube manufacturer's recommended volume  $\pm 5\%$  of Medical Air



**Analysis:** Pass the *Sample* through a carbon monoxide detector tube at the rate specified for the tube by the detector tube manufacturer.

**Acceptance criteria:** NMT 10 ppm

• **LIMIT OF SULFUR DIOXIDE**

**Sample:** Detector tube manufacturer's recommended volume  $\pm 5\%$  of Medical Air

**Analysis:** Pass the *Sample* through a sulfur dioxide detector tube at the rate specified for the tube by the detector tube manufacturer.

**Acceptance criteria:** NMT 5 ppm

• **LIMIT OF NITRIC OXIDE AND NITROGEN DIOXIDE**

**Sample:** Detector tube manufacturer's recommended volume  $\pm 5\%$  of Medical Air

**Analysis:** Pass the *Sample* through a nitric oxide–nitrogen dioxide detector tube at the rate specified for the tube by the detector tube manufacturer.

**Acceptance criteria:** NMT 2.5 ppm

• **LIMIT OF WATER AND OIL**

**Analysis:** Support one container in an inverted position (with the valve at the bottom) for 5 min. Cautiously open the valve slightly, maintaining the container in an inverted position. Vent the gas with a barely audible flow against a stainless steel mirror for a few seconds.

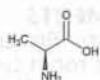
**Acceptance criteria:** No liquid is discernible on the mirror.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in pressurized containers. Container connections shall be appropriate for air. Adaptors shall not be used to connect containers to patient use supply system piping or equipment.

• **LABELING:** Label states if Medical Air is a synthetic mixture of *Oxygen USP* and *Nitrogen NF*. Where it is piped directly from the collecting tank to the patient point of use, label each outlet "Medical Air".

## Alanine



$C_3H_7NO_2$   
L-Alanine [56-41-7].

89.09

**DEFINITION**

Alanine contains NLT 98.5% and NMT 101.5% of L-alanine ( $C_3H_7NO_2$ ), calculated on the dried basis.

**IDENTIFICATION**

• **A. INFRARED ABSORPTION** (197K)

**ASSAY**

• **PROCEDURE**

**Sample:** 80 mg of Alanine

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Potentiometric

**Blank:** 3 mL of formic acid in 50 mL of glacial acetic acid

**Analysis:** Dissolve the *Sample* in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with *Titrant*.

Calculate the percentage of L-alanine ( $C_3H_7NO_2$ ) in the portion of Alanine taken:

$$\text{Result} = [(V_S - V_B) \times N \times F \times 100] / W$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 89.09 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 98.5%–101.5% on the dried basis

**IMPURITIES**

• **RESIDUE ON IGNITION** (281): NMT 0.15%

• **CHLORIDE AND SULFATE, Chloride** (221)

**Standard solution:** 0.70 mL of 0.020 N hydrochloric acid

**Sample:** 1.0 g of Alanine

**Acceptance criteria:** NMT 0.05%

• **CHLORIDE AND SULFATE, Sulfate** (221)

**Standard solution:** 0.30 mL of 0.020 N sulfuric acid

**Sample:** 1.0 g of Alanine

**Acceptance criteria:** NMT 0.03%

• **IRON** (241): NMT 30 ppm

**Delete the following:**

• **HEAVY METALS, Method I** (231): NMT 15 ppm • (Official 1-

Jan-2018)

• **RELATED COMPOUNDS**

**Mobile phase:** 0.008 N sulfuric acid solution

**System suitability solution:** A mixture of 0.05 mg/mL of USP Fumaric Acid RS, 0.05 mg/mL of USP Maleic Acid RS, and 3 mg/mL of USP Malic Acid RS in water

**Maleic acid standard solution:** 0.05 mg/mL of USP Maleic Acid RS in water

**Malic acid standard solution:** 3 mg/mL of USP Malic Acid RS in water

**Fumaric acid standard solution:** 0.05 mg/mL of USP Fumaric Acid RS in water

**Sample solution:** 100 mg/mL of Alanine in water

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 7.8-mm  $\times$  30-cm; 9- $\mu$ m packing L17

**Column temperature:** 30°

**Flow rate:** 0.6 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between maleic acid and malic acid

**Relative standard deviation:** NMT 5.0% for each of fumaric acid, maleic acid, and malic acid

**Analysis**

**Samples:** Standard solutions and *Sample solution*

Calculate the percentage of each specified acid in the portion of Alanine taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response of maleic acid, malic acid, or fumaric acid from the *Sample solution*

$r_S$  = peak response of maleic acid, malic acid, or fumaric acid from the corresponding Standard solution

$C_S$  = concentration of USP Maleic Acid RS, USP Malic Acid RS, or USP Fumaric Acid RS in the corresponding Standard solution (mg/mL)

$C_U$  = concentration of Alanine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Alanine taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity from the *Sample solution*



- $r_s$  = peak response of fumaric acid from the Fumaric acid standard solution  
 $C_s$  = concentration of USP Fumaric Acid RS in the Fumaric acid standard solution (mg/mL)  
 $C_u$  = concentration of Alanine in the Sample solution (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Maleic acid	0.5	0.05
Malic acid	0.6	0.05
Fumaric acid	1.0	0.05
Alanine	Not observed	—
Any unspecified impurity	—	0.05
Total unspecified impurities	—	0.20

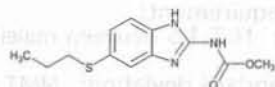
### SPECIFIC TESTS

- OPTICAL ROTATION, Specific Rotation (7815)**  
 Sample solution: 100 mg/mL in 6 N hydrochloric acid  
 Acceptance criteria: +13.7° to +15.1°
- PH (791):** 5.5–7.0, in a solution (1 in 20)
- LOSS ON DRYING (731)**  
 Analysis: Dry at 105° for 3 h.  
 Acceptance criteria: NMT 0.2%

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**  
 USP L-Alanine RS  
 USP Fumaric Acid RS  
 USP Maleic Acid RS  
 USP Malic Acid RS

## Albendazole



$C_{12}H_{15}N_3O_2S$  265.33  
 Carbamic acid, [5-(propylthio)-1H-benzimidazol-2-yl]-, methyl ester;  
 Methyl 5-(propylthio)-2-benzimidazolecarbamate [54965-21-8].

### DEFINITION

Albendazole contains NLT 98.0% and NMT 102.0% of albendazole ( $C_{12}H_{15}N_3O_2S$ ), calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION (197M)**
- B.** The  $R_f$  value of the principal spot of the Sample solution corresponds to that of the principal spot of the Standard solution, as obtained in the test for Organic Impurities.

### ASSAY

#### PROCEDURE

Sample: 250 mg of Albendazole  
 Analysis: Transfer the Sample to a suitable flask, and dissolve in 100 mL of glacial acetic acid, warming gen-

tly if necessary. Cool, and titrate with 0.1 N perchloric acid VS to a potentiometric endpoint (see *Titrimetry* (541)). Perform a blank determination. Each mL of 0.1 N perchloric acid is equivalent to 26.53 mg of  $C_{12}H_{15}N_3O_2S$ .

Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

- RESIDUE ON IGNITION (281):** NMT 0.2%
- ORGANIC IMPURITIES**  
 Standard stock solution: 5 mg/mL of USP Albendazole RS in glacial acetic acid  
 Standard solution: 0.05 mg/mL of USP Albendazole RS in glacial acetic acid from Standard stock solution  
 Sample solution: 10 mg/mL in glacial acetic acid  
**Chromatographic system**  
 (See *Chromatography* (621), *Thin-Layer Chromatography*.)  
**Mode:** TLC  
**Adsorbent:** 0.25-mm layer of silica gel mixture  
**Application volume:** 10  $\mu$ L  
**Developing solvent system:** Chloroform, ether, and glacial acetic acid (60:10:10)  
**Analysis:** Proceed as directed for *Chromatography* (621), *Thin-Layer Chromatography*.  
**Samples:** Standard stock solution, Standard solution, and Sample solution  
 Develop the chromatogram in the Developing solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow the solvent to evaporate from the plate, and examine the plate under short-wavelength UV light.  
 Acceptance criteria: 0.5%; no spot, other than the principal spot of the Sample solution, is larger or more intense than the principal spot of the Standard solution.

### SPECIFIC TESTS

- LOSS ON DRYING (731)**  
 Analysis: Dry at 105° for 4 h.  
 Acceptance criteria: NMT 0.5%

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**  
 USP Albendazole RS

## Albendazole Oral Suspension

### DEFINITION

Albendazole Oral Suspension is Albendazole in an aqueous vehicle. It contains one or more preservatives and dispersing or suspending agents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of albendazole ( $C_{12}H_{15}N_3O_2S$ ).

### IDENTIFICATION

- A. ULTRAVIOLET ABSORPTION (197U)**  
 Sample stock solution: 1 mg/mL of albendazole from a quantity of Suspension, in a mixture of methanol and hydrochloric acid (99:1). Filter the mixture, if necessary, to obtain a clear solution.  
 Sample solution: 0.01 mg/mL of albendazole in 0.1 N sodium hydroxide from Sample stock solution



Acceptance criteria: Meets the requirements

## ASSAY

### PROCEDURE

**Solution A:** Methanol and hydrochloric acid (99:1)

**Solution B:** 13.75 g/L of monobasic sodium phosphate

**Mobile phase:** Methanol and *Solution B* (60:40)

**Standard stock solution:** 1 mg/mL of USP Albendazole RS in *Solution A*

**Standard solution:** 100 µg/mL of USP Albendazole RS from *Standard stock solution* in *Mobile phase*

**Sample stock solution:** Equivalent to 1 mg/mL of albendazole from a volume of Oral Suspension in *Solution A*

**Sample solution:** Nominally 100 µg/mL of albendazole from *Sample stock solution* in *Mobile phase*. [NOTE—Filter, if necessary, to obtain a clear solution.]

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 308 nm

**Column:** 4-mm × 25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Column efficiency:** NLT 2000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of albendazole ( $C_{12}H_{15}N_3O_2S$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Albendazole RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of albendazole in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

## SPECIFIC TESTS

• **PH** <791>: 4.5–5.5

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **LABELING:** Label it to indicate that it is for veterinary use only.

• **USP REFERENCE STANDARDS** <11>

USP Albendazole RS

## Albendazole Tablets

» Albendazole Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of albendazole ( $C_{12}H_{15}N_3O_2S$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Labeling**—Tablets intended for veterinary use only are so labeled.

**USP Reference standards** <11>—

USP Albendazole RS

USP Parbendazole RS

## Identification—

**A: Ultraviolet Absorption** <197U>—

**Solution:** Dilute a portion of the clear filtrate used to prepare the *Assay preparation* and a portion of the stock solution used to prepare the *Standard preparation* prepared in the *Assay* with *Acidified methanol*, prepared as directed for *Dissolution*, to obtain solutions containing about 10 µg of albendazole per mL.

**B:** The retention time of the major peak for albendazole in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

## Dissolution

**Medium:** 0.1 N hydrochloric acid; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

Determine the amount of  $C_{12}H_{15}N_3O_2S$  dissolved using the following procedure.

**Acidified methanol**—To about 50 mL of methanol in a 100-mL volumetric flask add 2 mL of hydrochloric acid, dilute with methanol to volume, and mix.

**Standard solution**—Transfer about 90 mg of USP Albendazole RS, accurately weighed, to a 250-mL volumetric flask, add 10 mL of *Acidified methanol*, and shake to dissolve. Dilute with 0.1 N hydrochloric acid to volume, and mix. Transfer 5.0 mL of this solution to a 200-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.

**Procedure**—Transfer 10.0 mL of a filtered portion of the solution under test to a 250-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix. Concomitantly determine the absorbances of this solution and the *Standard solution* at the wavelengths of maximum and minimum absorbance at about 308 nm and 350 nm, using 0.1 N sodium hydroxide as the blank. Calculate the quantity, in mg, of  $C_{12}H_{15}N_3O_2S$  dissolved by the formula:

$$22.5C(A_U / A_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Albendazole RS in the *Standard solution*; and  $A_U$  and  $A_S$  are the differences in absorbance between 308 nm and 350 nm obtained from the solution under test and the *Standard solution*, respectively.

**Tolerances**—Not less than 80% ( $Q$ ) of the labeled amount of  $C_{12}H_{15}N_3O_2S$  is dissolved in 30 minutes.

**Uniformity of dosage units** <905>: meet the requirements.

**Procedure for content uniformity**—

**Acidified methanol and Standard solution**—Prepare as directed under *Dissolution*.

**Test solution**—Place 1 Tablet in a 500-mL volumetric flask, add about 300 mL of *Acidified methanol*, and shake by mechanical means for about 30 minutes. Dilute with *Acidified methanol* to volume, and mix. Filter a portion of this solution, discarding the first 20 mL of the filtrate. Transfer 4.0 mL of the clear filtrate to a 200-mL volumetric flask, dilute with 0.1 N sodium hydroxide to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the wavelengths of maximum and minimum absorbance at about 308 nm and 350 nm, using 0.1 N sodium hydroxide as the blank. Calculate the quantity, in mg, of  $C_{12}H_{15}N_3O_2S$  in the Tablet taken by the formula:

$$25C(A_U / A_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Albendazole RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the differences in absorbance between 308 nm and 350 nm obtained from the *Test solution* and the *Standard solution*, respectively.



**Assay—**

**Mobile phase**—Dissolve 0.50 g of monobasic ammonium phosphate in 400 mL of water. Add 600 mL of methanol, mix, and filter, discarding the first 15 mL of the filtrate. Degass the clear filtrate before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Sulfuric acid in methanol**—Prepare a mixture of 1 mL of sulfuric acid and 99 mL of methanol.

**Internal standard solution**—Transfer about 150 mg of USP Parbendazole RS to a 50-mL volumetric flask. Add 5 mL of *Sulfuric acid in methanol*, 25 mL of methanol, and shake to dissolve. Dilute with methanol to volume, and mix.

**Standard preparation**—Transfer about 100 mg of USP Albendazole RS, accurately weighed, to a 50-mL volumetric flask. Add 5 mL of *Sulfuric acid in methanol* and 25 mL of methanol, and shake to dissolve. Dilute with methanol to volume, and mix. Transfer 5.0 mL of this stock solution and 5.0 mL of *Internal standard solution* to a second 50-mL volumetric flask, dilute with methanol to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of albendazole, to a 50-mL volumetric flask. Add 5 mL of *Sulfuric acid in methanol* and 20 mL of methanol, and shake by mechanical means for about 15 minutes. Dilute with methanol to volume, mix, and filter, discarding the first 15 mL of the filtrate. Transfer 5.0 mL of the clear filtrate and 5.0 mL of *Internal standard solution* to a second 50-mL volumetric flask, dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; the column efficiency is not less than 1000 theoretical plates; the resolution between the albendazole peak and the parbendazole peak is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—[NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{12}H_{15}N_3O_2S$  in the portion of Tablets taken by the formula:

$$500C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Albendazole RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the albendazole peak to the parbendazole peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Albumin Human

**DEFINITION**

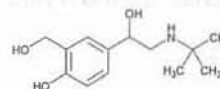
Albumin Human conforms to the regulations of the federal Food and Drug Administration concerning biologics (640.80 to 640.86) (see *Biologics* (1041)). It is a sterile, nonpyrogenic preparation of serum albumin obtained by fractionating material (source blood, plasma, serum, or placentas) from healthy human donors, the source material being tested for the absence of hepatitis B surface antigen. It is made by a process that yields a product that is safe for intravenous use. NLT 96% of its total protein is albumin. It is a solution containing, in each 100 mL, either 25 g of serum albumin osmotically equivalent to

500 mL of normal human plasma, or 20 g equivalent to 400 mL, or 5 g equivalent to 100 mL, or 4 g equivalent to 80 mL, and contains NLT 93.75% and NMT 106.25% of the labeled amount in the case of the solution containing 4 g in each 100 mL, and NLT 94.0% and NMT 106.0% of the labeled amount in the other cases. It contains no added antimicrobial agent, but may contain sodium acetyltryptophanate with or without sodium caprylate as a stabilizing agent. It has a sodium content of NLT 130 mEq/L and NMT 160 mEq/L. It has a heme content such that the absorbance of a solution, diluted to contain 1% of protein, in a 1-cm holding cell, measured at a wavelength of 403 nm, is NMT 0.25. It meets the requirements of the test for heat stability and for pH.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at the temperature recommended by the manufacturer or indicated on the label.
- **EXPIRATION DATE:** The expiration date is not later than 5 years after issue from manufacturer's cold storage (5°, 3 years) if labeling recommends storage between 2° and 10°; not later than 3 years after issue from manufacturer's cold storage (5°, 3 years) if labeling recommends storage at temperatures not higher than 37°; and not later than 10 years after date of manufacture if in a hermetically sealed metal container and labeling recommends storage between 2° and 10°.
- **LABELING:** Label it to state that it is not to be used if it is turbid and that it is to be used within 4 h after the container is entered. Label it also to state the osmotic equivalent in terms of plasma, the sodium content, and the type of source material (venous plasma, placental plasma, or both) from which it was prepared. Label it also to indicate that additional fluids are needed when the 20-g/100-mL or 25-g/100-mL product is administered to a markedly dehydrated patient.

## Albuterol



$C_{13}H_{21}NO_3$  239.31  
1,3-Benzenedimethanol,  $\alpha$ 1-[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-;  
 $\alpha$ 1-[(tert-Butylamino)methyl]-4-hydroxy-m-xylene- $\alpha,\alpha'$ -diol  
[18559-94-9].

**DEFINITION**

Albuterol contains NLT 98.5% and NMT 101.0% of albuterol ( $C_{13}H_{21}NO_3$ ), calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B. ULTRAVIOLET ABSORPTION (197U)**  
Sample solution: 80 μg/mL in 0.1 N hydrochloric acid  
Acceptance criteria: Meets the requirements

**ASSAY**

- **PROCEDURE**  
Sample solution: 8 mg/mL of Albuterol in glacial acetic acid  
Analysis: To 50 mL of the *Sample solution* add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 23.93 mg of  $C_{13}H_{21}NO_3$ .  
Acceptance criteria: 98.5%–101.0% on the anhydrous basis



**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

- **ORGANIC IMPURITIES**

**Standard solution:** 0.10 mg/mL of USP Albuterol RS in methanol

**Sample solution:** 20 mg/mL of Albuterol in methanol

**Chromatographic system**  
(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Methyl isobutyl ketone, isopropyl alcohol, ethyl acetate, ammonium hydroxide, and water (50:45:35:3:18)

**Visualization:** Iodine vapor

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in the chapter, applying aliquots of the *Standard solution* and the *Sample solution*. Develop in the *Developing solvent system* until the solvent front has moved three-fourths the length of the plate. Remove the plate from the developing chamber, air-dry, and expose it to iodine vapor.

**Acceptance criteria:** Any spot, other than the principal spot, obtained from the *Sample solution* is not greater in size and intensity than the spot produced by the *Standard solution* (0.5%), and the sum of the impurities is not greater than 2.0%.

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method I** (921): NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Albuterol RS

**Albuterol Tablets****DEFINITION**

Albuterol Tablets contain an amount of albuterol sulfate [(C<sub>13</sub>H<sub>21</sub>NO<sub>3</sub>)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub>] equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of albuterol (C<sub>13</sub>H<sub>21</sub>NO<sub>3</sub>).

**IDENTIFICATION**

- **A.** The *R<sub>f</sub>* value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*, obtained as directed in the *Procedure for Organic Impurities*.
- **B. IDENTIFICATION TESTS—GENERAL, Sulfate** (191)  
**Sample solution:** Shake a quantity of powdered Tablets equivalent to 4 mg of albuterol with 10 mL of water, and filter. Use the filtrate.  
**Acceptance criteria:** Meet the requirements

**ASSAY**

- **PROCEDURE**

**Solution A:** 10 mL/L of glacial acetic acid in water

**Solution B:** 1.13 g of sodium 1-hexanesulfonate in 1200 mL of water. Add 12 mL of glacial acetic acid.

**Diluent:** Methanol and water (40:60)

**Mobile phase:** Methanol and *Solution B* (40:60)

**Standard stock solution:** 0.12 mg/mL of USP Albuterol Sulfate RS, prepared as follows. Transfer USP Albuterol Sulfate RS to a suitable volumetric flask, and add a volume of *Solution A* corresponding to 60% of the flask volume. Sonicate for 5 min, and dilute with methanol to volume.

**Standard solution:** 0.03 mg/mL of USP Albuterol Sulfate RS in *Diluent*, from *Standard stock solution*

**Sample solution:** Nominally 0.025 mg/mL of albuterol, prepared as follows. Transfer a number of whole Tablets, equivalent to 50 mg of albuterol, to a suitable volumetric flask. Add 60% of the flask volume of *Solution A*, shake by mechanical means for 45 min, sonicate for 10 min, allow to cool to room temperature, and dilute with methanol to volume. Pass through a suitable filter of 0.45- $\mu$ m or finer pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 276 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 25  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 800 theoretical plates

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of albuterol (C<sub>13</sub>H<sub>21</sub>NO<sub>3</sub>) in the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times M \times (M_{r1}/M_{r2}) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of albuterol in the *Sample solution* (mg/mL)

*M* = number of moles of albuterol per mole of albuterol sulfate, 2

*M<sub>r1</sub>* = molecular weight of albuterol, 239.31

*M<sub>r2</sub>* = molecular weight of albuterol sulfate, 576.70

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **DISSOLUTION, Procedure for a Pooled Sample** (711)

**Medium:** Water; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Diluent, Mobile phase, and Standard stock solution:**  
Proceed as directed in the *Assay*.

**Standard solution:** 0.03 mg/mL of USP Albuterol Sulfate RS in *Diluent*, from *Standard stock solution*. If necessary, dilute with *Diluent* to a concentration corresponding to the *Sample solution*.

**Sample solution:** Pass a portion of the solution under test through a nylon filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 276 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 100  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 800 theoretical plates

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of albuterol (C<sub>13</sub>H<sub>21</sub>NO<sub>3</sub>) dissolved by comparing the major peak response from the *Sample solution* to that from the *Standard solution*.

**Acceptance criteria:** NLT 80% (Q) of the labeled amount of albuterol (C<sub>13</sub>H<sub>21</sub>NO<sub>3</sub>) is dissolved.



- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES**• **ORGANIC IMPURITIES**

**Standard solution A:** 0.580 mg/mL of USP Albuterol Sulfate RS in water, equivalent to 0.483 mg/mL of albuterol

**Standard solution B:** 0.218 mg/mL of USP Albuterol Sulfate RS in water, equivalent to 0.183 mg/mL of albuterol

**Standard solution C:** 0.073 mg/mL of USP Albuterol Sulfate RS in water, equivalent to 0.061 mg/mL of albuterol

**Sample solution:** Place a quantity of finely powdered Tablets, equivalent to 48 mg of albuterol, into a suitable container. Add 60 mL of diluted alcohol (1 in 2), and shake by mechanical means for 30 min. Filter the mixture, and wash the filter with small portions of alcohol, combining this with the filtrate. Evaporate the filtrate to dryness under reduced pressure below 40°. Dissolve the residue as completely as possible in 2 mL of water.

**Chromatographic system**

(See Chromatography (621), Thin-Layer Chromatography.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 10 µL. Apply two successive 5-µL aliquots, allowing the solvent to evaporate between applications.

**Developing solvent system:** Methyl isobutyl ketone, isopropyl alcohol, ethyl acetate, ammonium hydroxide, and water (50:45:35:3:18)

**Spray reagent A:** 3-Methyl-2-benzothiazolinone hydrazone hydrochloride TS

**Spray reagent B:** Ammoniacal potassium ferricyanide TS

**Analysis**

**Samples:** *Standard solution A, Standard solution B, Standard solution C, and Sample solution*

Air-dry the plate. Develop the chromatograms until the solvent front has moved about 17 cm. Spray the plate first with *Spray reagent A*, and then *Spray reagent B*, and finally again with *Spray reagent A*. Examine the plate and estimate the responses of any secondary spots observed in the lane of the *Sample solution* by comparison with those of *Standard solutions A, B, and C*.

**Acceptance criteria**

1. 2.0%; no major secondary spot is greater in size or intensity than the principal spot produced by *Standard solution A*.
2. 0.75%; no other secondary spot is greater in size or intensity than the principal spot produced by *Standard solution B*.
3. 0.25%; no more than two other secondary spots are equal in size or intensity to the principal spot produced by *Standard solution C*.
4. The sum of the intensities of all secondary spots obtained from the *Sample solution* corresponds to NMT 3.5%.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

- **USP REFERENCE STANDARDS (11)**  
USP Albuterol Sulfate RS

**Albuterol Extended-Release Tablets****DEFINITION**

Albuterol Extended-Release Tablets contain albuterol sulfate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of albuterol ( $C_{13}H_{21}NO_3$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B. ULTRAVIOLET ABSORPTION (197U)**  
**Standard solution:** 80 µg/mL of albuterol from USP Albuterol Sulfate RS in methanol  
**Sample solution:** 80 µg/mL of albuterol in methanol, prepared as follows. Transfer a suitable number of Tablets to a volumetric flask, and dilute with methanol to volume. Stir for 30 min, and centrifuge.  
**Wavelength range:** 210–350 nm  
**Cell path:** 0.2 cm  
**Acceptance criteria:** The *Sample solution* exhibits maxima and minima only at the same wavelengths as the *Standard solution*.

**ASSAY**• **PROCEDURE**

**Buffer:** 0.65 g/L of sodium 1-octane sulfonate and 21.7 g/L of ammonium acetate in water

**Mobile phase:** Glacial acetic acid, 2-propanol, methanol, and *Buffer* (4:3:1:92)

**Diluent:** 10 mL/L of triethylamine in water

**Standard stock solution:** 0.2 mg/mL of USP Albuterol Sulfate RS in *Diluent*

**Standard solution:** 0.02 mg/mL of USP Albuterol Sulfate RS in *Diluent*, from the *Standard stock solution*.

Transfer an aliquot of the *Standard stock solution* to a suitable volumetric flask, and add 4% of the flask volume of methanol. Allow to cool to room temperature, and dilute with *Diluent* to volume.

**Sample solution:** Nominally 0.016 mg/mL of albuterol, prepared as follows. Transfer Tablets (NLT 10) to a suitable volumetric flask, add 10% of the flask volume of methanol, and sonicate for 30 min with regular swirling. Add 60% of the flask volume of *Diluent*, and sonicate for 30 min with swirling. Stir for 60 min, allow the solution to cool to room temperature, and dilute with *Diluent* to volume. Centrifuge a portion of this solution at 2500 rpm for 15 min. Transfer 10 mL of the supernatant into a 50-mL volumetric flask, add 1 mL of methanol, cool to room temperature, and dilute with *Diluent* to volume. Pass the solution through a 1-µm glass fiber or equivalent filter, and discard the first 3 mL of the filtrate.

**Chromatographic system**

(See Chromatography (621), System Suitability.)



Mode: LC

Detector: UV 276 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 40 μL

System suitability

Sample: Standard solution

Suitability requirements

Column efficiency: NLT 2000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of albuterol ( $C_{13}H_{21}NO_3$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M \times M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Albuterol Sulfate RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of albuterol in the Sample solution (mg/mL)

$M$  = moles of albuterol per mole of albuterol sulfate, 2

$M_{r1}$  = molecular weight of albuterol, 239.31

$M_{r2}$  = molecular weight of albuterol sulfate, 576.70

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm, with helix sinker

Time: 1, 2, 4, and 9 h

Buffer, Mobile phase, Chromatographic system, and

System suitability: Proceed as directed in the Assay, except to use an Injection volume of 50 μL.

Standard stock solution: 0.2 mg/mL of albuterol, using USP Albuterol Sulfate RS, in Medium

Standard solution: Dilute the Standard stock solution with Medium to obtain a final concentration of (L/1000) mg/mL of albuterol, where L is the label claim in mg/Tablet of albuterol.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Analysis

Samples: Standard solution and Sample solution

Calculate the concentration ( $C_i$ ) of albuterol ( $C_{13}H_{21}NO_3$ ) in the sample withdrawn from the vessel at each time point (i):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of albuterol in the Standard solution (mg/mL)

Calculate the percentage of the labeled amount of albuterol ( $C_{13}H_{21}NO_3$ ) released at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_3)] + [C_1 \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times (V - (2 \times V_3))] + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times (V - (3 \times V_3))] + [(C_3 + C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$C_i$  = concentration of albuterol in the portion of sample withdrawn at time point i (mg/mL)

$V$  = volume of the Medium (900 mL)

$L$  = label claim (mg/Tablet)

$V_3$  = volume of the Sample solution withdrawn from the Medium (mL)

Tolerances: See Table 1.

Table 1

Time point (i)	Time (h)	Amount released (%)
1	1	25–45
2	2	45–65
3	4	65–85
4	9	NLT 80

The cumulative percentages of the labeled amount of albuterol ( $C_{13}H_{21}NO_3$ ), released at the times specified, conform to Acceptance Table 2 in Dissolution (711).

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES, PROCEDURE 1

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with hydrochloric acid to a pH of 3.0.

Mobile phase: Methanol and Buffer (5:95)

System suitability solution: 2 μg/mL each of USP Albuterol Sulfate RS and USP Albuterol Related Compound B RS in Mobile phase

Standard solution: 2.4 μg/mL of USP Albuterol Sulfate RS, 0.80 μg/mL of USP Levalbuterol Related Compound C RS, and 0.25 μg/mL of USP Levalbuterol Related Compound D RS (equivalent to 0.20 μg/mL free base) in Mobile phase

Sensitivity solution: 0.06 μg/mL of USP Albuterol Sulfate RS in Mobile phase

Sample solution: Transfer an amount of powder from NLT 20 Tablets to a suitable volumetric flask to obtain a solution with a final nominal concentration of 0.2 mg/mL of albuterol. Add 70% of the flask volume of Mobile phase, and sonicate for 10 min. Stir for 30 min, and dilute with Mobile phase to volume. Pass the solution through a 1-μm glass fiber or equivalent filter, and discard the first 3 mL of the filtrate.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; 5-μm packing L11

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 80 μL

Run time: 5 times the retention time of albuterol

System suitability

Samples: System suitability solution and Standard solution

[NOTE—Identify the impurities using the relative retention times shown in Table 2.]

Suitability requirements:

Tailing factor: NMT 2.0 for each compound, Standard solution

Resolution: NLT 2 between albuterol and albuterol related compound B, System suitability solution

Relative standard deviation: NMT 10.0% for each compound, Standard solution

Analysis

Samples: Standard solution, Sensitivity solution, and Sample solution

[NOTE—Identify the impurities using the relative retention times shown in Table 2.]



Calculate the percentage of levalbuterol related compound C in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of levalbuterol related compound C from the *Sample solution*  
 $r_S$  = peak response of levalbuterol related compound C from the *Standard solution*  
 $C_S$  = concentration of USP Levalbuterol Related Compound C RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of albuterol in the *Sample solution* (mg/mL)

Calculate the percentage of levalbuterol related compound D in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of levalbuterol related compound D from the *Sample solution*  
 $r_S$  = peak response of the levalbuterol related compound D from the *Standard solution*  
 $C_S$  = concentration of USP Levalbuterol Related Compound D RS (as the free base) in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of albuterol in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M \times M_{r1}/M_{r2}) \times 100$$

- $r_U$  = peak response of the impurity from the *Sample solution*  
 $r_S$  = peak response of albuterol from the *Standard solution*  
 $C_S$  = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of albuterol in the *Sample solution* (mg/mL)  
 $M$  = moles of albuterol per mole of albuterol sulfate, 2  
 $M_{r1}$  = molecular weight of albuterol, 239.31  
 $M_{r2}$  = molecular weight of albuterol sulfate, 576.70

**Acceptance criteria:** See Table 2. Disregard peaks eluting after levalbuterol related compound C or with areas less than that of the *Sensitivity solution*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Albuterol related compound B <sup>a</sup>	0.88	— <sup>b</sup>
Albuterol	1.0	—
Chloroalbuterone <sup>c</sup>	1.7	— <sup>b</sup>
Chloroalbuterol <sup>d</sup>	2.5	— <sup>b</sup>
Albuterol related compound A <sup>e</sup>	2.7	— <sup>b</sup>

<sup>a</sup> 2-(*tert*-Butylamino)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone.

<sup>b</sup> Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

<sup>c</sup> 2-(*tert*-Butylamino)-1-[3-chloro-4-hydroxy-5-(hydroxymethyl)phenyl]ethanone.

<sup>d</sup> 4-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-chloro-6-(hydroxymethyl)phenol.

<sup>e</sup> 4-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-methylphenol.

<sup>f</sup> 5-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxy-benzaldehyde.

<sup>g</sup> α-[(1,1-Dimethylethyl)amino]methyl]-4-hydroxy-3-(methoxymethyl)-benzenemethanol.

<sup>h</sup> Disregard peaks eluting after levalbuterol related compound C.

Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Levalbuterol related compound D <sup>f</sup>	3.2	0.1
Levalbuterol related compound C <sup>g,h</sup>	3.5	0.4
Any other individual unspecified impurity	—	0.2

<sup>a</sup> 2-(*tert*-Butylamino)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone.

<sup>b</sup> Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

<sup>c</sup> 2-(*tert*-Butylamino)-1-[3-chloro-4-hydroxy-5-(hydroxymethyl)phenyl]ethanone.

<sup>d</sup> 4-[2-(*tert*-Butylamino)-1-hydroxyethyl]-2-chloro-6-(hydroxymethyl)phenol.

<sup>e</sup> 4-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-methylphenol.

<sup>f</sup> 5-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxy-benzaldehyde.

<sup>g</sup> α-[(1,1-Dimethylethyl)amino]methyl]-4-hydroxy-3-(methoxymethyl)-benzenemethanol.

<sup>h</sup> Disregard peaks eluting after levalbuterol related compound C.

#### • ORGANIC IMPURITIES, PROCEDURE 2

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with hydrochloric acid to a pH of 3.0.

**Mobile phase:** Methanol and *Buffer* (30:70)

**Diluent:** Methanol and *Buffer* (5:95)

**Peak identification solution:** 2.4 µg/mL of USP Albuterol Sulfate RS, 1.0 µg/mL of USP Levalbuterol Related Compound C RS, and 1.2 µg/mL of USP Levalbuterol Related Compound D RS in *Diluent*

**Standard solution:** 2.4 µg/mL of USP Albuterol Sulfate RS and 1.0 µg/mL of USP Albuterol Related Compound E RS in *Diluent*

**Sensitivity solution:** 0.06 µg/mL of USP Albuterol Sulfate RS in *Diluent* from *Standard solution*

**Sample solution:** Transfer an amount of powder from NLT 20 Tablets to a suitable volumetric flask to obtain a solution with a final concentration of 0.2 mg/mL of albuterol. Add 70% of the flask volume of *Diluent*, and sonicate for 10 min. Stir for 30 min, and dilute with *Diluent* to volume. Pass the solution through a glass fiber or equivalent filter of 1-µm pore size, and discard the first 3 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 225 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L11

**Flow rate:** 0.7 mL/min

**Injection volume:** 80 µL

**Run time:** 5 times the retention time of albuterol

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for each compound

**Relative standard deviation:** NMT 10.0% for each compound

#### Analysis

**Samples:** *Standard solution*, *Peak identification solution*, *Sensitivity solution*, and *Sample solution*

Calculate the percentage of albuterol related compound E in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of albuterol related compound E from the *Sample solution*  
 $r_S$  = peak response of albuterol related compound E from the *Standard solution*



$C_S$  = concentration of USP Albuterol Related Compound E RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of albuterol in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M \times M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of the impurity from the *Sample solution*

$r_S$  = peak response of albuterol from the *Standard solution*

$C_S$  = concentration of USP Albuterol Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of albuterol in the *Sample solution* (mg/mL)

$M$  = moles of albuterol per mole of albuterol sulfate, 2

$M_{r1}$  = molecular weight of albuterol, 239.31

$M_{r2}$  = molecular weight of albuterol sulfate, 576.70

**Acceptance criteria:** See Table 3. Disregard the levalbuterol related compound C peak and any peak eluting before it. Disregard peaks with areas less than that of the *Sensitivity solution*.

Table 3

Compound	Relative Retention Time	Acceptance Criteria, NMT (%)
Albuterol	1.0	—
Levalbuterol related compound C	1.79	—
Levalbuterol related compound D	1.83	—
Albuterol related compound E <sup>a</sup>	3.67	0.5
Any other individual unspecified impurity	—	0.2
Total impurities	—	1.5 <sup>b</sup>

<sup>a</sup> 2,2'-Oxybis(methylene)bis[4-[2-(*tert*-butylamino)-1-hydroxyethyl]phenol].

<sup>b</sup> From the sum of the impurities in Procedure 1 and Procedure 2.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

#### • USP REFERENCE STANDARDS (11)

USP Albuterol Sulfate RS

USP Albuterol Related Compound B RS

2-(*tert*-Butylamino)-1-[4-hydroxy-3-(hydroxymethyl)phenyl]ethanone.

$C_{13}H_{19}NO_3$  237.29

USP Albuterol Related Compound E RS

2,2'-Oxybis(methylene)bis[4-[2-(*tert*-butylamino)-1-hydroxyethyl]phenol].

$C_{26}H_{40}N_2O_5$  460.61

USP Levalbuterol Related Compound C RS

$\alpha$ -[[(1,1-Dimethylethyl)amino]methyl]-4-hydroxy-3-(methoxymethyl)-benzenemethanol.

$C_{14}H_{23}NO_3$  253.34

USP Levalbuterol Related Compound D RS

5-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxy-benzaldehyde sulfate salt.

$(C_{13}H_{19}NO_3)_2 \cdot H_2SO_4$  572.67

### Albuterol Sulfate

$(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$  576.70

1,3-Benzenedimethanol,  $\alpha$ '-[[[(1,1-dimethylethyl)amino]methyl]-4-hydroxy-, sulfate (2:1) (salt).

$\alpha$ '-[[[(*tert*-Butylamino)methyl]-4-hydroxy-*m*-xylene- $\alpha, \alpha'$ -diol sulfate (2:1) (salt) [51022-70-9].

» Albuterol Sulfate contains not less than 98.5 percent and not more than 101.0 percent of  $(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

#### USP Reference standards (11)—

USP Albuterol Related Compound A RS

4-[2-[(1,1-Dimethylethyl)amino]-1-hydroxyethyl]-2-methylphenol sulfate.

USP Albuterol Sulfate RS

#### Identification—

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

*Solution:* 80  $\mu$ g per mL.

*Medium:* 0.1 N hydrochloric acid.

**C:** Shake a quantity of it, equivalent to 4 mg of albuterol, with 10 mL of water, and filter: the filtrate so obtained meets the requirements of the tests for *Sulfate* (191).

**D:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water Determination, Method I** (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity**—It meets the requirements of the test for *Organic Impurities* under *Albuterol*, except to read Albuterol Sulfate in place of Albuterol and to use water instead of methanol as the solvent to prepare the *Standard solution* and the *Sample solution*.

#### Assay—

**0.05  $\pm$  0.01 M Ammonium acetate solution**—Dissolve 3.85 g of ammonium acetate in 1000 mL of water, and mix.

**Mobile phase**—Prepare a degassed mixture of water, 0.05  $\pm$  0.01 M Ammonium acetate solution, and isopropanol [65:30: (5  $\pm$  1)], and adjust dropwise with acetic acid to a pH of 4.5  $\pm$  0.3.

**Resolution solution**—Dissolve accurately weighed quantities of USP Albuterol Sulfate RS and USP Albuterol Related Compound A RS in water, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.140 mg per mL and 0.030 mg per mL, respectively.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Albuterol Sulfate RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 0.6 mg per mL.

**Assay preparation**—Transfer about 60 mg of Albuterol Sulfate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 276-nm detector and a 4.6-mm  $\times$  20-cm column that contains packing L10. The flow rate is about 2.0 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between albuterol and albuterol related compound A is not less than 1.5; and the relative standard deviation for replicate injections is not more than 1.5%.

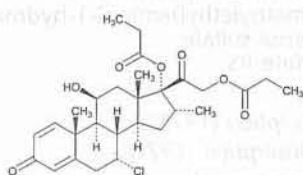


**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $(C_{13}H_{21}NO_3)_2 \cdot H_2SO_4$  in the portion of Albuterol Sulfate taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Albuterol Sulfate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Alclometasone Dipropionate



$C_{28}H_{37}ClO_7$  521.04  
Pregna-1,4-diene-3,20-dione, 7-chloro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (7 $\alpha$ ,11 $\beta$ ,16 $\alpha$ )-; 7 $\alpha$ -Chloro-11 $\beta$ ,17,21-trihydroxy-16 $\alpha$ -methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [66734-13-2].

### DEFINITION

Alclometasone Dipropionate contains NLT 97.0% and NMT 102.0% of  $C_{28}H_{37}ClO_7$ , calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197M)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the *Internal standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Solution A:** 6.80 mg/mL of monobasic potassium phosphate (0.05 M)

**Mobile phase:** Methanol and *Solution A* (2:1)

**Internal standard solution:** 2 mg/mL of betamethasone dipropionate in methanol

**Standard stock solution:** 1.2 mg/mL of USP Alclometasone Dipropionate RS in methanol

**Standard solution:** 4.0 mL of *Standard stock solution* and 4.0 mL of *Internal standard solution*. Dilute with methanol to 25 mL. [NOTE—This solution contains approximately 0.2 mg/mL of USP Alclometasone Dipropionate RS.]

**Sample stock solution:** 1.2 mg/mL of Alclometasone Dipropionate in methanol

**Sample solution:** 4 mL of *Sample stock solution* and 4 mL of *Internal standard solution*. Dilute with methanol to 25 mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm  $\times$  30-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for alclometasone dipropionate and betamethasone dipropionate are about 0.7 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 3.0 between the analyte and the internal standard peaks

**Relative standard deviation:** NMT 2%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{28}H_{37}ClO_7$  in the portion of Alclometasone Dipropionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak height ratio from the *Sample solution*

$R_S$  = peak height ratio from the *Standard solution*

$C_S$  = concentration of USP Alclometasone Dipropionate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

### IMPURITIES

#### Inorganic Impurities

- RESIDUE ON IGNITION** (281): NMT 0.1%

#### Delete the following:

- HEAVY METALS, Method II** (231): NMT 30 ppm (Official 1.

Jan-2018)

#### Organic Impurities

##### PROCEDURE

**Mobile phase:** Acetonitrile and water (3:2)

**Diluent:** Acetonitrile and water (2:1)

**System suitability solution:** 1.5 mg/mL of USP Alclometasone Dipropionate RS and 0.015 mg/mL of USP Alclometasone Dipropionate Related Compound A RS in *Diluent*

**Sample solution:** 1.5 mg/mL of Alclometasone Dipropionate in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection size:** 5  $\mu$ L

**Run time:** Three times the retention time of alclometasone

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Tailing factor:** NMT 1.5 for alclometasone dipropionate

**Relative standard deviation:** NMT 2.0% for alclometasone dipropionate

**Resolution:** NLT 2.0 between alclometasone dipropionate and alclometasone dipropionate related compound A

### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Alclometasone Dipropionate taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

$r_U$  = peak area for each impurity from the *Sample solution*

$r_T$  = sum of all the peaks from the *Sample solution*

*F* = relative response factor (see *Impurity Table 1*)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 2.0%



Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alclometasone dipropionate	1.0	—	—
Alclometasone dipropionate related compound A <sup>a</sup>	1.2	0.93	1.0
2-Bromo alclometasone dipropionate <sup>b</sup>	1.7	0.91	0.5
Any individual, unspecified impurity	—	1.0	0.10

<sup>a</sup> 11 $\beta$ ,17,21-Trihydroxy-16 $\alpha$ -methylpregna-1,4-diene-3,20-dione 17,21-dipropionate.

<sup>b</sup> 2-Bromo-7 $\alpha$ -chloro-11 $\beta$ ,17,21-trihydroxy-16 $\alpha$ -methylpregna-1,4-diene-3,20-dione 17,21-dipropionate.

### SPECIFIC TESTS

- OPTICAL ROTATION, Specific Rotation (781S)**  
Sample solution: 30 mg/mL in dioxane  
Acceptance criteria: +21° to +25°
- LOSS ON DRYING (731):** Dry a sample in a vacuum at a pressure not exceeding 5 mm of mercury at 105° for 3 h; it loses NMT 0.5% of its weight.

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**  
USP Alclometasone Dipropionate RS  
USP Alclometasone Dipropionate Related Compound A RS  
11 $\beta$ ,17,21-Trihydroxy-16 $\alpha$ -methylpregna-1,4-diene-3,20-dione 17,21-dipropionate.  
C28H38O7 486.60

## Alclometasone Dipropionate Cream

### DEFINITION

Alclometasone Dipropionate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of alclometasone dipropionate (C28H37ClO7) in a suitable cream base.

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.
- B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**  
**Standard solution:** 0.08 mg/mL of USP Alclometasone Dipropionate RS in methanol  
**Sample solution:** Place a quantity of Cream, equivalent to 1.25 mg of alclometasone dipropionate, in a 50-mL centrifuge tube, and add 15 mL of methanol. Insert a stopper securely into the tube, and place the tube in a water bath maintained at 60° until the semisolid components melt. Remove the tube from the bath, shake vigorously until the specimen components resolidify, and place the tube in an ice-methanol bath for 15 min. Remove the tube from the bath, and centrifuge at 2500 rpm for 5 min. Transfer the clear supernatant to a vial, and allow to equilibrate to room temperature.

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 20  $\mu$ L

**Developing solvent system:** Chloroform and acetone (7:1)

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Dry the applications with the aid of a stream of nitrogen, and develop the chromatograms in a saturated, unlined chromatographic chamber. When the solvent front has moved three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot obtained from the *Sample solution* corresponds to that of the *Standard solution*.

### ASSAY

#### PROCEDURE

**Buffer:** 6.80 g/L of monobasic potassium phosphate (0.05 M)

**Mobile phase:** Methanol and *Buffer* (2:1)

**Internal standard solution:** 0.4 mg/mL of betamethasone dipropionate in methanol

**Standard stock solution:** 0.25 mg/mL of USP Alclometasone Dipropionate RS in methanol

**Standard solution:** 0.08 mg/mL of USP Alclometasone Dipropionate RS obtained by combining, in a small stoppered flask, 5.0 mL of *Standard stock solution*, 5.0 mL of methanol, and 5.0 mL of *Internal standard solution*

**Sample solution:** Transfer a quantity of Cream, equivalent to 1.25 mg of alclometasone dipropionate, to a 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution* and 10.0 mL of methanol. Insert a stopper securely into the tube, and place it in a water bath maintained at 60° until the semisolid components melt. Remove the tube from the bath, shake vigorously until the specimen components resolidify, and return the tube to the 60° water bath until the semisolid components melt. Remove the tube from the bath, shake vigorously until the specimen components resolidify, and place the tube in an ice-methanol bath for 15 min. Remove the tube from the bath, and centrifuge at 2500 rpm for 5 min. Transfer the clear supernatant to a small stoppered flask, and allow to equilibrate to room temperature.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm  $\times$  30-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for alclometasone dipropionate and betamethasone dipropionate are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between the analyte and internal standard peaks

**Relative standard deviation:** NMT 2%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alclometasone dipropionate (C28H37ClO7) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak height ratio of alclometasone dipropionate to the internal standard from the *Sample solution*

$R_S$  = peak height ratio of alclometasone dipropionate to the internal standard from the *Standard solution*



- $C_s$  = concentration of USP Alclometasone Dipropionate RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of alclometasone dipropionate in the *Sample solution* (mg/mL)  
 Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**

- **MINIMUM FILL (755):** Meets the requirements

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Alclometasone Dipropionate RS

## Alclometasone Dipropionate Ointment

**DEFINITION**

Alclometasone Dipropionate Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of alclometasone dipropionate ( $C_{28}H_{37}ClO_7$ ) in a suitable ointment base.

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the *Assay*.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 0.25 mg/mL USP Alclometasone Dipropionate RS in methanol

**Sample solution:** Place a quantity of Ointment, equivalent to 1.25 mg of alclometasone dipropionate, in a 50-mL centrifuge tube, add 10 mL of 2,2,4-trimethylpentane, insert a stopper securely into the tube, and disperse the specimen using a vortex mixer. Add 5.0 mL of a solution of methanol in water (45 in 50), insert the stopper securely, shake vigorously for 2 min, and centrifuge at 2500 rpm for 3 min. Remove the lower, aqueous alcohol phase, and transfer to a stoppered vial.

**Chromatographic system**  
(See *Chromatography (621)*, *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 20  $\mu$ L

**Developing solvent system:** Chloroform and acetone (7:1)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Dry the applications with the aid of a stream of nitrogen, and develop the chromatograms in a saturated, unlined chromatographic chamber. When the solvent front has moved three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot obtained from the *Sample solution* corresponds to that of the *Standard solution*.

**ASSAY**• **PROCEDURE**

**Buffer:** 6.80 g/L of monobasic potassium phosphate (0.05 M)

**Solution A:** Dilute 450 mL of methanol with water to 500 mL.

**Mobile phase:** Methanol and *Buffer* (2:1)

**Internal standard solution:** 0.15 mg/mL of

betamethasone dipropionate in *Solution A*

**Standard stock solution:** 0.1 mg/mL of USP Alclometasone Dipropionate RS in *Solution A*

**Standard solution:** 0.05 mg/mL of USP Alclometasone

Dipropionate RS obtained by combining, in a small stoppered flask, 5.0 mL of *Standard stock solution* and 5.0 mL of *Internal standard solution*

**Sample solution:** Transfer a quantity of Ointment, equivalent to 0.5 mg of alclometasone dipropionate, to a 50-mL centrifuge tube, add 10 mL of 2,2,4-trimethylpentane, insert a stopper securely into the tube, and disperse the specimen using a vortex mixer. Add 5.0 mL of *Internal standard solution* and 5.0 mL of *Solution A*, insert the stopper securely, shake vigorously for 2 min, and centrifuge at 2500 rpm for 3 min. Remove the lower, aqueous alcohol phase, and transfer this *Sample solution* to a stoppered vial.

**Chromatographic system**

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm  $\times$  30-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for alclometasone dipropionate and betamethasone dipropionate are about 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.0 between the analyte and internal standard peaks

**Relative standard deviation:** NMT 2%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alclometasone dipropionate ( $C_{28}H_{37}ClO_7$ ) in the portion of Ointment taken:

$$\text{Result} = (R_u/R_s) \times (C_s/C_u) \times 100$$

$R_u$  = peak height ratio of alclometasone dipropionate to the internal standard from the *Sample solution*

$R_s$  = peak height ratio of alclometasone dipropionate to the internal standard from the *Standard solution*

$C_s$  = concentration of USP Alclometasone Dipropionate RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of alclometasone dipropionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**

- **MINIMUM FILL (755):** Meets the requirements

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Alclometasone Dipropionate RS

**Alcohol**

Portions of this monograph that are national USP text, and are not part of the harmonized text, are marked with symbols (♦) to specify this fact.



$\text{C}_2\text{H}_6\text{O}$  46.07  
Ethanol;  
Ethyl alcohol [64-17-5].

**DEFINITION**

♦Alcohol contains NLT 92.3% and NMT 93.8%, by weight, corresponding to NLT 94.9% and NMT 96.0%, by volume, at 15.56°, of  $\text{C}_2\text{H}_5\text{OH}$ .♦

**IDENTIFICATION**

- **A.** It meets the requirements of the test for *Specific Gravity* (841).
- **B. INFRARED ABSORPTION** (197F) or (197S): Neat

**IMPURITIES**• **LIMIT OF NONVOLATILE RESIDUE**

Sample: 100 mL of Alcohol

Analysis: Evaporate the Sample in a tared dish on a water bath, and dry at 100°–105° for 1 h.

Acceptance criteria: The weight of the residue is NMT 2.5 mg.

• **ORGANIC IMPURITIES**

Sample solution A: Alcohol (substance under test)

Sample solution B: 300 µL/L of 4-methylpentan-2-ol in Sample solution A

Standard solution A: 200 µL/L of methanol in Sample solution A

Standard solution B: 10 µL/L of methanol and 10 µL/L of acetaldehyde in Sample solution A

Standard solution C: 30 µL/L of acetal in Sample solution A

Standard solution D: 2 µL/L of benzene in Sample solution A

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m fused-silica capillary; bonded with a 1.8-µm layer of phase G43

Split ratio: 20:1

Temperatures

Injection port: 200°

Detector: 280°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	12
40	10	240	10

Linear velocity: 35 cm/s

Carrier gas: Helium

Injection volume: 1.0 µL

**System suitability**

Sample: Standard solution B

**Suitability requirements**

Resolution: NLT 1.5 between the first major peak (acetaldehyde) and the second major peak (methanol)

**Analysis**

Samples: Sample solution A, Sample solution B, Standard solution A, Standard solution B, Standard solution C, and Standard solution D

**Methanol calculation**

$$\text{Result} = (r_U/r_S)$$

$r_U$  = peak area of methanol from Sample solution A

$r_S$  = peak area of methanol from Standard solution A

**Acetaldehyde calculation** (sum of acetaldehyde and acetal)

$$\text{Result} = \{[A_E/(A_T - A_E)] \times C_A\} + \{[D_E/(D_T - D_E)] \times C_D \times (M_{r1}/M_{r2})\}$$

$A_E$  = peak area of acetaldehyde from Sample solution A

$A_T$  = peak area of acetaldehyde from Standard solution B

$C_A$  = concentration of acetaldehyde in Standard solution B (µL/L)

$D_E$  = peak area of acetal from Sample solution A

$D_T$  = peak area of acetal from Standard solution C

$C_D$  = concentration of acetal in Standard solution C (µL/L)

$M_{r1}$  = molecular weight of acetaldehyde, 44.05

$M_{r2}$  = molecular weight of acetal, 118.2

**Benzene calculation**

$$\text{Result} = [B_E/(B_T - B_E)] \times C_B$$

$B_E$  = peak area of benzene from Sample solution A

$B_T$  = peak area of benzene from Standard solution D

$C_B$  = concentration of benzene in Standard solution D (µL/L)

[NOTE—If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).]

**Any other impurity calculation**

$$\text{Result} = (r_U/r_M) \times C_M$$

$r_U$  = peak area of each impurity in Sample solution B

$r_M$  = peak area of 4-methylpentan-2-ol in Sample solution B

$C_M$  = concentration of 4-methylpentan-2-ol in Sample solution B (µL/L)

Acceptance criteria: See Table 2.

Table 2

Name	Acceptance Criteria
Methanol	NMT 0.5, corresponding to 200 µL/L
Acetaldehyde and acetal	NMT 10 µL/L, expressed as acetaldehyde

♦ Disregard any peaks of less than 9 µL/L (0.03 times the area of the peak corresponding to 4-methylpentan-2-ol in the chromatogram obtained with Sample solution B).



Table 2 (Continued)

Name	Acceptance Criteria
Benzene	NMT 2 $\mu\text{L/L}$
Sum of all other impurities <sup>a</sup>	NMT 300 $\mu\text{L/L}$

<sup>a</sup> Disregard any peaks of less than 9  $\mu\text{L/L}$  (0.03 times the area of the peak corresponding to 4-methylpentan-2-ol in the chromatogram obtained with *Sample solution B*).

**SPECIFIC TESTS**

- **\*SPECIFIC GRAVITY (841):** 0.812–0.816 at 15.56°, indicating 92.3%–93.8%, by weight, or 94.9%–96.0%, by volume, of  $\text{C}_2\text{H}_5\text{OH}$ .

- **ULTRAVIOLET ABSORPTION**

Analytical wavelength: 235–340 nm

Cell: 5 cm

Reference: Water

Acceptance criteria

Absorbance: NMT 0.40 at 240 nm; NMT 0.30 between 250 nm and 260 nm; NMT 0.10 between 270 nm and 340 nm

Curve: The spectrum shows a steadily descending curve with no observable peaks or shoulders.

- **\*CLARITY OF SOLUTION**

[NOTE—The *Sample solution* is to be compared to *Standard suspension A* and to water in diffused daylight 5 min after preparation of *Standard suspension A*.]

**Hydrazine solution:** 10 mg/mL of hydrazine sulfate in water. Allow to stand for 4–6 h.

**Methenamine solution:** Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

**Primary opalescent suspension:** Transfer 25.0 mL of *Hydrazine solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

**Opalescence standard:** Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. This suspension should not be used beyond 24 h after preparation.

**Standard suspension A:** *Opalescence standard* and water (1 in 20)

**Standard suspension B:** *Opalescence standard* and water (1 in 10)

**Sample solution A:** Substance to be examined

**Sample solution B:** Dilute 1.0 mL of *Sample solution A* with water to 20 mL, and allow to stand for 5 min before testing.

**Blank:** Water

**Analysis:** Transfer a sufficient portion of *Sample solution A* and *Sample solution B* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension A*, *Standard suspension B*, and *Blank* to separate matching test tubes. Compare *Sample solution A*, *Sample solution B*, *Standard suspension A*, *Standard suspension B*, and *Blank* in diffused daylight, viewing vertically against a black background (see *Nephelometry, Turbidimetry, and Visual Comparison* (855), *Visual Comparison*). The diffusion of light must be such that *Standard suspension A* can readily be distinguished from water, and *Standard suspension B* can readily be distinguished from *Standard suspension A*.

**Acceptance criteria:** *Sample solution A* and *Sample solution B* show the same clarity as that of water or their opalescence is not more pronounced than that of *Standard suspension A*.

**ACIDITY OR ALKALINITY**

**Phenolphthalein solution:** Dissolve 0.1 g of phenolphthalein in 80 mL of alcohol, and dilute with water to 100 mL.

**Sample:** 20 mL of Alcohol

**Analysis:** To the *Sample* add 20 mL of freshly boiled and cooled water and 0.1 mL of *Phenolphthalein solution*. The solution is colorless. Add 1.0 mL of 0.01 N sodium hydroxide.

**Acceptance criteria:** The solution is pink (30  $\mu\text{L/L}$ , expressed as acetic acid).

- **\*COLOR OF SOLUTION**

**Standard stock solution:** Combine 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, 2.4 mL of cupric sulfate CS, and 1.6 mL of dilute hydrochloric acid (10 g/L).

**Standard solution:** Transfer 1.0 mL of *Standard stock solution* to a 100-mL volumetric flask, and dilute with dilute hydrochloric acid (10 g/L). Prepare the *Standard solution* immediately before use.

**Sample solution:** Substance to be examined

**Blank:** Water

**Analysis:** Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of the *Standard solution* and *Blank* to separate, matching test tubes. Compare the *Sample solution*, *Standard solution*, and *Blank* in diffused daylight, viewing vertically against a white background (see *Nephelometry, Turbidimetry, and Visual Comparison* (855), *Visual Comparison*).

**Acceptance criteria:** The *Sample solution* has the appearance of water or is not more intensely colored than the *Standard solution*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.

- **USP REFERENCE STANDARDS (11)**  
USP Alcohol RS

**Dehydrated Alcohol**

Portions of this monograph that are national USP text, and are not part of the harmonized text, are marked with symbols (♦) to specify this fact.



$\text{C}_2\text{H}_6\text{O}$

Ethanol;

Ethyl alcohol [64-17-5].

46.07

**DEFINITION**

- ♦ Dehydrated Alcohol contains NLT 99.2% by weight, corresponding to NLT 99.5% by volume, at 15.56°, of  $\text{C}_2\text{H}_5\text{OH}$ .

**IDENTIFICATION**

- **A.** It meets the requirements of the test for *Specific Gravity* (841).
- **B. INFRARED ABSORPTION (197S) or (197F):** Neat

**IMPURITIES**

- **LIMIT OF NONVOLATILE RESIDUE**

**Sample:** 100 mL of Dehydrated Alcohol

**Analysis:** Evaporate the *Sample* in a tared dish on a water bath, and dry at 100°–105° for 1 h.

**Acceptance criteria:** The weight of the residue is NMT 2.5 mg.



# • **ORGANIC IMPURITIES**

**Sample solution A:** Substance to be examined

**Sample solution B:** 300  $\mu\text{L/L}$  of 4-methylpentan-2-ol in *Sample solution A*

**Standard solution A:** 200  $\mu\text{L/L}$  of methanol in *Sample solution A*

**Standard solution B:** 10  $\mu\text{L/L}$  of methanol and 10  $\mu\text{L/L}$  of acetaldehyde in *Sample solution A*

**Standard solution C:** 30  $\mu\text{L/L}$  of acetal in *Sample solution A*

**Standard solution D:** 2  $\mu\text{L/L}$  of benzene in *Sample solution A*

## **Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.32-mm  $\times$  30-m fused-silica capillary; bonded with a 1.8- $\mu\text{m}$  layer of phase G43

**Split ratio:** 20:1

**Temperatures**

**Injection port:** 200°

**Detector:** 280°

**Column:** See *Table 1*.

**Table 1**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	12
40	10	240	10

**Flow rate:** 35 cm/s

**Carrier gas:** Helium

**Injection volume:** 1.0  $\mu\text{L}$

## **System suitability**

**Sample:** *Standard solution B*

**Suitability requirements**

**Resolution:** NLT 1.5 between the first major peak (acetaldehyde) and the second major peak (methanol)

## **Analysis**

**Samples:** *Sample solution A*, *Sample solution B*, *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Standard solution D*

**Methanol calculation**

$$\text{Result} = r_U/r_S$$

$r_U$  = peak area of methanol from *Sample solution A*

$r_S$  = peak area of methanol from *Standard solution A*

**Acetaldehyde calculation** (sum of acetaldehyde and acetal)

$$\text{Result} = \{[A_E/(A_T - A_E)] \times C_A\} + \{[D_E/(D_T - D_E)] \times C_D \times (M_{r1}/M_{r2})\}$$

$A_E$  = peak area of acetaldehyde from *Sample solution A*

$A_T$  = peak area of acetaldehyde from *Standard solution B*

$C_A$  = concentration of acetaldehyde in *Standard solution B* ( $\mu\text{L/L}$ )

$D_E$  = peak area of acetal from *Sample solution A*

$D_T$  = peak area of acetal from *Standard solution C*

$C_D$  = concentration of acetal in *Standard solution C* ( $\mu\text{L/L}$ )

$M_{r1}$  = molecular weight of acetaldehyde, 44.05

$M_{r2}$  = molecular weight of acetal, 118.2

**Benzene calculation**

$$\text{Result} = [B_E/(B_T - B_E)] \times C_B$$

$B_E$  = peak area of benzene from *Sample solution A*

$B_T$  = peak area of benzene from *Standard solution D*

$C_B$  = concentration of benzene in *Standard solution D* ( $\mu\text{L/L}$ )

[NOTE—If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).]

**Any other impurity calculation**

$$\text{Result} = (r_U/r_M) \times C_M$$

$r_U$  = peak area of each impurity from *Sample solution B*

$r_M$  = peak area of 4-methylpentan-2-ol from *Sample solution B*

$C_M$  = concentration of 4-methylpentan-2-ol in *Sample solution B* ( $\mu\text{L/L}$ )

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Acceptance Criteria
Methanol	NMT 0.5, corresponding to 200 $\mu\text{L/L}$
Acetaldehyde and acetal	NMT 10 $\mu\text{L/L}$ , expressed as acetaldehyde
Benzene	NMT 2 $\mu\text{L/L}$
Sum of all other impurities <sup>a</sup>	NMT 300 $\mu\text{L/L}$

<sup>a</sup> Disregard any peaks of less than 9  $\mu\text{L/L}$  (0.03 times the area of the peak corresponding to 4-methylpentan-2-ol in the chromatogram obtained with *Sample solution B*).

## **SPECIFIC TESTS**

• **\*SPECIFIC GRAVITY** (841): NMT 0.7962 at 15.56°, indicating NLT 99.2% of  $\text{C}_2\text{H}_5\text{OH}$  by weight.

• **ULTRAVIOLET ABSORPTION**

**Analytical wavelength:** 235–340 nm

**Cell:** 5 cm

**Reference:** Water

**Acceptance criteria**

**Absorbance:** NMT 0.40 at 240 nm; NMT 0.30 between 250 and 260 nm; NMT 0.10 between 270 and 340 nm

**Curve:** The spectrum shows a steadily descending curve with no observable peaks or shoulders.

• **\*CLARITY OF SOLUTION**

[NOTE—The *Sample solution* is to be compared to *Standard suspension A* and to water in diffused daylight 5 min after preparation of *Standard suspension A*.]

**Hydrazine solution:** 10 mg/mL of hydrazine sulfate in water. Allow to stand for 4–6 h.

**Methenamine solution:** Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

**Primary opalescent suspension:** Transfer 25.0 mL of *Hydrazine solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.

**Opalescence standard:** Transfer 15.0 mL of the *Primary opalescent suspension* to a 1000-mL volumetric flask, and dilute with water to volume. This suspension should not be used beyond 24 h after preparation.

**Standard suspension A:** Dilute 5.0 mL of the *Opalescence standard* with water to 100.0 mL.

**Standard suspension B:** Dilute 10.0 mL of the *Opalescence standard* with water to 100.0 mL.



**Sample solution A:** Substance to be examined

**Sample solution B:** 1.0 mL of *Sample solution A* diluted with water to 20 mL. Allow to stand for 5 min before testing.

**Blank:** Water

#### Analysis

**Samples:** *Standard suspension A*, *Standard suspension B*, *Sample solution A*, *Sample solution B*, and *Blank*

Transfer a sufficient portion of *Sample solution A* and *Sample solution B* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension A*, *Standard suspension B*, and *Blank* to separate matching test tubes. Compare samples in diffused daylight, viewing vertically against a black background (see *Nephelometry*, *Turbidimetry*, and *Visual Comparison* (855), *Visual Comparison*). The diffusion of light must be such that *Standard suspension A* can be readily distinguished from water, and *Standard suspension B* can be readily distinguished from *Standard suspension A*.

**Acceptance criteria:** *Sample solution A* and *Sample solution B* show the same clarity as that of water, or their opalescence is not more pronounced than that of *Standard suspension A*.

#### • ACIDITY OR ALKALINITY

**Phenolphthalein solution:** Dissolve 0.1 g of phenolphthalein in 80 mL of alcohol, and dilute with water to 100 mL.

**Sample:** 20 mL of Dehydrated Alcohol

**Analysis:** To the *Sample* add 20 mL of freshly boiled and cooled water and 0.1 mL of *Phenolphthalein solution*. The solution is colorless. Add 1.0 mL of 0.01 N sodium hydroxide.

**Acceptance criteria:** The solution is pink (30 µg/g, expressed as acetic acid).

#### • COLOR OF SOLUTION

**Standard stock solution:** Combine 3.0 mL of ferric chloride CS, 3.0 mL of cobaltous chloride CS, 2.4 mL of cupric sulfate CS, and 1.6 mL of dilute hydrochloric acid (10 mg/mL).

**Standard solution:** 1.0 mL of *Standard stock solution*, diluted with dilute hydrochloric acid (10 mg/mL) to 100 mL. Prepare the *Standard solution* immediately before use.

**Sample solution:** Substance to be examined

**Blank:** Water

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*

Transfer a sufficient portion of each of the *Samples* to individual test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Compare the *Samples* in diffused daylight, viewing vertically against a white background (see *Nephelometry*, *Turbidimetry*, and *Visual Comparison* (855), *Visual Comparison*).

**Acceptance criteria:** The *Sample solution* has the appearance of water or is not more intensely colored than the *Standard solution*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light.

#### • USP REFERENCE STANDARDS (11)

USP Dehydrated Alcohol RS

### Dehydrated Alcohol Injection

» Dehydrated Alcohol Injection is Dehydrated Alcohol suitable for parenteral use.

**Packaging and storage—**Preserve in tight, single-dose containers, preferably of Type I glass, and store at controlled room temperature. The container may contain an inert gas in the headspace.

#### Identification—

**A:** Mix 5 drops in a small beaker with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid, and cover the beaker immediately with a filter paper moistened with a solution recently prepared by dissolving 0.1 g of sodium nitroferricyanide and 0.25 g of piperazine in 5 mL of water: an intense blue color is produced on the filter paper, the color becoming paler after a few minutes.

**B:** To 5 mL of a solution (1 in 10) add 1 mL of 1.0 N sodium hydroxide, then slowly (over a period of 3 minutes) add 2 mL of 0.1 N iodine: the odor of iodoform develops, and a yellow precipitate is formed within 30 minutes.

**Specific gravity** (841): not more than 0.8035 at 15.5°, indicating not less than 96.8%, by weight, of  $C_2H_5OH$ .

**Acidity—**To 50 mL, in a glass-stoppered flask, add 50 mL of recently boiled water. Add phenolphthalein TS, and titrate with 0.020 N sodium hydroxide to a pink color that persists for 30 seconds: not more than 10.0 mL of 0.020 N sodium hydroxide is required for neutralization.

**Limit of nonvolatile residue—**Evaporate 40 mL in a tared dish on a water bath, and dry at 105° for 1 hour: the weight of the residue does not exceed 1 mg.

**Water-insoluble substances—**Dilute it with an equal volume of water: the mixture is clear and remains clear for 30 minutes after cooling to 10°.

**Aldehydes and other foreign organic substances—**Place 20 mL in a glass-stoppered cylinder that has been thoroughly cleaned with hydrochloric acid, then rinsed with water and finally with the dehydrated alcohol to be tested. Cool the contents to approximately 15°, and add, by means of a carefully cleaned pipet, 0.10 mL of 0.10 N potassium permanganate, noting accurately the time of addition. Mix at once by inverting the stoppered cylinder, and allow it to stand at 15° for 5 minutes: the pink color does not entirely disappear.

**Amyl alcohol and nonvolatile, carbonizable substances—**Allow 25 mL to evaporate spontaneously from a porcelain dish, carefully protected from dust, until the surface of the dish is barely moist: no red or brown color is produced immediately upon the addition of a few drops of sulfuric acid.

**Ultraviolet absorbance—**Record the UV absorption spectrum between 340 nm and 235 nm in a 1-cm cell, with water in a matched cell in the reference beam: the absorbance is not more than 0.08 at 240 nm, and 0.02 between 270 nm and 340 nm, and the curve drawn through these points is smooth.

**Limit of acetone and isopropyl alcohol—**To 1.0 mL add 1 mL of water, 1 mL of a saturated solution of dibasic sodium phosphate, and 3 mL of a saturated solution of potassium permanganate. Warm the mixture to 45° to 50°, and allow to stand until the permanganate color is discharged. Add 3 mL of 2.5 N sodium hydroxide, and pass, without washing, through a sintered-glass filter. Prepare a control containing 1 mL of the saturated solution of dibasic sodium



phosphate, 3 mL of 2.5 N sodium hydroxide, and 80 µg of acetone in 9 mL. To each solution add 1 mL of furfural solution (1 in 100), and allow to stand for 10 minutes, then to 1.0 mL of each solution add 3 mL of hydrochloric acid: any pink color produced in the test solution is not more intense than that in the control.

**Methanol**—To 1 drop add 1 drop of water, 1 drop of dilute phosphoric acid (1 in 20), and 1 drop of potassium permanganate solution (1 in 20). Mix, allow to stand for 1 minute, and add sodium metabisulfite solution (1 in 20), dropwise, until the permanganate color is discharged. If a brown color remains, add 1 drop of the dilute phosphoric acid. To the colorless solution add 5 mL of freshly prepared chromotropic acid TS, and heat on a water bath at 60° for 10 minutes: no violet color appears.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

## Alcohol in Dextrose Injection

### DEFINITION

Alcohol in Dextrose Injection is a sterile solution of Alcohol and Dextrose in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of alcohol (C<sub>2</sub>H<sub>5</sub>OH), and NLT 95.0% and NMT 105.0% of the labeled amount of dextrose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> · H<sub>2</sub>O).

### IDENTIFICATION

- **A.** **Sample solution:** A few drops of Injection (1 in 20) in water  
**Analysis:** Add the *Sample solution* to 5 mL of hot alkaline cupric tartrate TS.  
**Acceptance criteria:** A copious red precipitate of cuprous oxide is formed.
- **B. SPECIFIC GRAVITY (841):** NMT 0.7962 at 15.56°, indicating NLT 99.2% of alcohol (C<sub>2</sub>H<sub>5</sub>OH) by weight
- **C. INFRARED ABSORPTION (197S) or (197F):** Meets the requirements

### ASSAY

- **ALCOHOL DETERMINATION, Method 1—Distillation Method (611)**

**Sample solution:** 50.0 mL  
**Acceptance criteria:** 90.0%–110.0%

- **DEXTROSE**

**Sample:** Equivalent to 2–5 g of dextrose from a suitable volume of injection

**Analysis:** Transfer the *Sample solution* to a 100-mL volumetric flask, add 0.2 mL of 6 N ammonium hydroxide, and dilute with water to volume. Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* (781)).

Calculate the percentage of dextrose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> · H<sub>2</sub>O) in the portion of Injection taken:

$$\text{Result} = [(M_{r1}/M_{r2})/R_{mid}]AR \times 100$$

$M_{r1}$  = molecular weight of dextrose monohydrate, 198.17

$M_{r2}$  = molecular weight of anhydrous dextrose, 180.16

$R_{mid}$  = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

$A$  = 100 mm divided by the length of the polarimeter tube (mm)

$R$  = observed rotation (°)

**Acceptance criteria:** 95.0%–105.0%

### IMPURITIES

**Delete the following:**

- **HEAVY METALS (231)**

**Test preparation:** Equivalent to 4.0 g of dextrose from a volume of Injection

**Analysis:** Transfer the *Sample* to a vessel, and adjust the volume to 25 mL by evaporation or by addition of water, as necessary.

**Acceptance criteria:** NMT 5 × C ppm, where C is the labeled amount, in g, of dextrose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> · H<sub>2</sub>O) per mL of Injection (Official 1-Jan-2018)

- **LIMIT OF 5-HYDROXYMETHYLFURFURAL AND RELATED SUBSTANCES**

**Sample solution:** Equivalent to 2 mg/mL of dextrose in water from a suitable volume of Injection

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** 284 nm

**Cell:** 1 cm

**Blank:** Water

**Acceptance criteria:** The absorbance is NMT 0.25.

### SPECIFIC TESTS

- **pH (791):** 3.5–6.5

**Sample solution:** A portion of Injection to which 0.30 mL of saturated potassium chloride solution has been added for each 100 mL and which previously has been diluted with water, if necessary, to a concentration of NMT 5% of dextrose

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.5 USP Endotoxin unit/mL

- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, single-dose containers, preferably of Type I or Type II glass, and store at controlled room temperature.

- **LABELING:** The label states the total osmolality of the solution expressed in mOsmol/L.

- **USP REFERENCE STANDARDS (11)**  
USP Endotoxin RS

## Rubbing Alcohol

### DEFINITION

Rubbing Alcohol and all preparations under the classification of Rubbing Alcohols are manufactured in accordance with the requirements of the U.S. Treasury Department, Bureau of Alcohol, Tobacco, and Firearms, Formula 23-H (8 parts by volume of acetone, 1.5 parts by volume of methyl isobutyl ketone, and 100 parts by volume of ethyl alcohol) being used. It contains NLT 68.5% and NMT 71.5% by volume of dehydrated alcohol, the remainder consisting of water and the denaturants, with or without color additives, and perfume oils. Rubbing Alcohol contains, in each 100 mL, NLT 355 mg of sucrose octaacetate or NLT 1.40 mg of denatonium benzoate. The preparation may be colored with one or more color additives, listed by the FDA for use in drugs. A suitable stabilizer may be added. Rubbing Alcohol complies with the requirements of the Bureau of Alcohol, Tobacco, and Firearms of the U.S. Treasury Department.

[NOTE—Rubbing Alcohol is packaged, labeled, and sold in accordance with the regulations issued by the U.S. Treasury Department, Bureau of Alcohol, Tobacco, and Firearms.]



**ASSAY**• **DENATONIUM BENZOATE**

**Buffer:** 9.23 g of anhydrous dibasic sodium phosphate in 800 mL of water. Adjust with saturated citric acid solution to a pH of  $4 \pm 0.1$ , dilute with water to 1000 mL, and mix.

**Standard solution:** 50 µg/mL of USP Denatonium Benzoate RS in water

**Sample solution:** Dissolve the residue obtained in the test for *Limit of Nonvolatile Residue* in 50.0 mL of water, and transfer to a suitable flask.

**Instrumental conditions**

**Analytical wavelength:** Maximum absorbance at about 410 nm

**Cell:** 1 cm

**Analysis**

**Samples:** *Buffer*, *Standard solution*, and *Sample solution* Transfer 10.0 mL each of *Buffer*, *Standard solution*, and *Sample solution* to individual 250-mL separators. Add to each 40 mL of *Buffer* 10 mL of a 1-in-1000 solution of bromophenol blue in chloroform and 60 mL of chloroform. Shake the separators vigorously for 2 min, allow to stand for 15 min, then withdraw the chloroform layers through chloroform-washed cotton into 100-mL volumetric flasks. Repeat the extraction with 20 mL of chloroform, adding the filtered chloroform extracts to the respective volumetric flasks, and dilute with chloroform to volume. Without delay, concomitantly determine the absorbances of the solutions, using the blank to set a suitable spectrophotometer. Calculate the quantity, in mg, of denatonium benzoate ( $C_{28}H_{34}N_2O_3 \cdot H_2O$ ) in 100 mL of Rubbing Alcohol:

$$\text{Result} = (A_U/A_S) \times C_S \times 0.025$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Denatonium Benzoate RS in the *Standard solution* (µg/mL)

**Acceptance criteria:** NLT 1.40 mg

• **SUCROSE OCTAACETATE**

**Sample solution:** Using about 50 mL of 70% alcohol, transfer the residue obtained in the test for *Limit of Nonvolatile Residue* to a 500-mL conical flask.

**Analysis:** Neutralize the *Sample solution* with 0.1 N sodium hydroxide VS, using phenolphthalein TS as the indicator. Add 25.0 mL of 0.1 N sodium hydroxide, attach an air condenser to the flask, and reflux on a steam bath for 1 h. Remove from the steam bath, cool quickly, and titrate the excess alkali with 0.1 N sulfuric acid VS, using phenolphthalein TS as the indicator. Perform a blank determination (see *Titrimetry* <541>, *Residual Titrations*). Each mL of 0.1 N sodium hydroxide is equivalent to 8.483 mg of sucrose octaacetate ( $C_{28}H_{38}O_{19}$ ).

**Acceptance criteria:** NLT 355 mg of sucrose octaacetate per 100 mL of Rubbing Alcohol

**IMPURITIES**• **METHANOL**

**Sample solution:** Dilute 0.50 mL of Rubbing Alcohol with water to 1.0 mL.

**Analysis:** To 0.50 mL of the *Sample solution* add 1 drop of dilute phosphoric acid (1 in 20) and 1 drop of potassium permanganate solution (1 in 20). Mix, allow to stand for 1 min, and add dropwise sodium metabisulfite solution (1 in 20) until the permanganate color is discharged. If a brown color remains, add 1 drop of dilute phosphoric acid (1 in 20). To the colorless solution add 5 mL of freshly prepared chromotropic acid TS, and heat in a water bath at 60° for 10 min.

**Acceptance criteria:** No violet color appears.

**SPECIFIC TESTS**

• **SPECIFIC GRAVITY** <841>: 0.8691–0.8771 at 15.56° (the U.S. government standard temperature for alcohol determination) for Rubbing Alcohol manufactured with specially denatured alcohol Formula 23-H

• **LIMIT OF NONVOLATILE RESIDUE**

Where the denaturant is denatonium benzoate

**Sample:** 200.0 mL of Rubbing Alcohol

**Analysis:** Evaporate the *Sample*, transferred in convenient portions, in a suitable tared dish on a steam bath, and dry the residue at 105° for 1 h. Retain the residue for the *Assay for Denatonium Benzoate*.

**Acceptance criteria:** The weight of the residue is NLT 2.8 mg.

Where the denaturant is sucrose octaacetate

**Sample:** 25.0 mL of Rubbing Alcohol

**Analysis:** Evaporate the *Sample* in a suitable tared dish on a steam bath, and dry the residue at 105° for 1 h. Retain the residue for the *Assay for Sucrose Octaacetate*.

**Acceptance criteria:** The weight of the residue is NLT 89 mg.

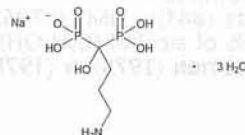
**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers, remote from fire, and store at controlled room temperature.

• **LABELING:** Label it to indicate that it is flammable.

• **USP REFERENCE STANDARDS** (11)

USP Denatonium Benzoate RS

**Alendronate Sodium**

$C_{12}H_{12}NNaO_7P_2 \cdot 3H_2O$

325.12

Phosphonic acid, (4-amino-1-hydroxybutylidene) bis-, monosodium salt, trihydrate;

Sodium trihydrogen (4-amino-1-hydroxybutylidene)diphosphonate, trihydrate [121268-17-5].

**DEFINITION**

Alendronate Sodium contains NLT 98.0% and NMT 102.0% of alendronate sodium ( $C_{12}H_{12}NNaO_7P_2$ ), calculated on the dried basis.

**IDENTIFICATION**

• **A. INFRARED ABSORPTION** <197M>

• **B. IDENTIFICATION TESTS—GENERAL, Sodium** <191>: Meets the requirements of the pyroantimonate precipitation test

**ASSAY**• **PROCEDURE**

**Buffer solution:** 14.7 g/L of sodium citrate dihydrate and 7.05 g/L of anhydrous dibasic sodium phosphate. Adjust with phosphoric acid to a pH of 8.

**Mobile phase:** Acetonitrile, methanol, and *Buffer solution* (25:5:70)

**Diluent:** 29.4 g/L of sodium citrate dihydrate

**Borate solution:** 19.1 g/L of sodium borate

**Solution A:** 0.5 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [NOTE—Prepare this solution fresh just before use.]

**Standard stock solution:** 0.1 mg/mL of USP Alendronate Sodium RS in *Diluent*



**Standard solution:** Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of *Solution A*, and shake for 30 s. Allow to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min. Use a portion of the clear upper aqueous layer.

**Reagent blank:** Transfer 5.0 mL of *Diluent* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of *Solution A*, and shake for 30 s. Allow to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min. Use a portion of the clear upper aqueous layer.

**Sample stock solution:** 0.1 mg/mL of Alendronate Sodium in *Diluent*

**Sample solution:** Transfer 5.0 mL of the *Sample stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of *Solution A*, and shake for 30 s. Allow to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min. Use a portion of the clear upper aqueous layer.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 266 nm

**Column:** 4.1-mm × 25-cm; packing L21

**Column temperature:** 35°

**Flow rate:** 1.2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0% for replicate injections

#### Analysis

**Samples:** *Standard solution*, *Reagent blank*, and *Sample solution*

Calculate the percentage of alendronate sodium ( $C_4H_{12}NNaO_7P_2$ ) in the portion of Alendronate Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Alendronate Sodium RS in the *Standard stock solution* (mg/mL)

$C_U$  = concentration of Alendronate Sodium in the *Sample stock solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

#### IMPURITIES

##### Delete the following:

• **HEAVY METALS, Method III (231):** 0.001% (Official 1-Jan-2018)

##### • ORGANIC IMPURITIES

**Buffer solution:** 2.94 g/L of sodium citrate dihydrate and 1.42 g/L of anhydrous dibasic sodium phosphate. Adjust with phosphoric acid to a pH of 8 and pass through a filter of 0.5-µm or finer pore size.

**Solution A:** Acetonitrile and *Buffer solution* (3:17)

**Solution B:** Acetonitrile and *Buffer solution* (7:3)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	50	50
25	0	100
27	100	0
32	100	0

**Diluent and Borate solution:** Proceed as directed in the Assay.

**Solution C:** 4 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [NOTE—Prepare this solution fresh just before use.]

**Standard stock solution:** 0.6 mg/mL of USP Alendronate Sodium RS in *Diluent*

**Standard solution A:** Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.

**Standard solution B:** 0.6 µg/mL of USP Alendronate Sodium RS in *Diluent* from *Standard stock solution*. Transfer 5 mL of this diluted solution (0.6 µg/mL) to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.

**Reagent blank:** Transfer 5.0 mL of *Diluent* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.

**Sample stock solution:** 0.6 mg/mL of Alendronate Sodium in *Diluent*

**Sample solution:** Transfer 5.0 mL of *Sample stock solution* to a 50-mL polypropylene, screw-cap centrifuge tube containing 5 mL of *Borate solution*. Add 5 mL of acetonitrile and 5 mL of *Solution C*, and shake for 45 s. Allow to stand at room temperature for 30 min. Add 20 mL of methylene chloride, and shake vigorously for 1 min. Centrifuge for 5–10 min, and use a portion of the clear upper aqueous layer.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 266 nm

**Column:** 4.1-mm × 25-cm; packing L21

**Column temperature:** 45°

**Flow rate:** 1.8 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *Standard solution A* and *Standard solution B*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for the main peak, *Standard solution A*

**Signal-to-noise ratio:** NLT 3 for the main peak, *Standard solution B*

#### Analysis

**Samples:** *Reagent blank* and *Sample solution*

[NOTE—Disregard any peak corresponding to those obtained from the *Reagent blank*.]



Calculate the percentage of each impurity in the portion of Alendronate Sodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak area of each impurity

$r_T$  = sum of all impurity peaks and the main peak

#### Acceptance criteria

Individual impurities: NMT 0.1%

Total impurities: NMT 0.5%

#### SPECIFIC TESTS

##### • LOSS ON DRYING (731)

Sample: Dry at a pressure of NMT 5 mm of mercury at 140° to constant weight.

Acceptance criteria: 16.1%–17.1%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.

##### • USP REFERENCE STANDARDS (11)

USP Alendronate Sodium RS

## Alendronate Sodium Tablets

#### DEFINITION

Alendronate Sodium Tablets contain an amount of Alendronate Sodium equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of alendronic acid ( $C_4H_7NO_7P_2$ ).

#### IDENTIFICATION

• The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Solution A:** 14.7 g/L of sodium citrate dihydrate and 7.05 g/L of anhydrous dibasic sodium phosphate in water. [NOTE—Adjust with phosphoric acid to a pH of 8.0 before bringing the solution to volume.]

**Solution B:** 38.1 g/L of sodium borate in water

**Solution C:** 1 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [NOTE—Prepare this solution fresh just before use.]

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (20:5:75)

**Diluent:** 29.4 g/L of sodium citrate dihydrate in water

**Standard stock solution:** 0.03 mg/mL of anhydrous alendronate sodium in *Diluent*, from USP Alendronate Sodium RS

**Standard solution:** Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.

**Sample stock solution:** Transfer NLT 10 Tablets to a 1000-mL volumetric flask. Add 500 mL of *Diluent*, shake by mechanical means for 30 min, and sonicate for 5 min. Dilute with *Diluent* to volume, and centrifuge a portion of this solution. Quantitatively dilute a portion of the clear supernatant to a concentration of 0.02–0.03 mg/mL of alendronic acid.

**Sample solution:** Transfer 5.0 mL of the *Sample stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add

25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.

**Blank:** Transfer 5 mL of *Diluent* to a 50-mL polypropylene screw-cap centrifuge tube containing 5 mL of *Solution B*, and mix for 3 min. Add 4 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 10 min. Use the clear upper aqueous layer.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 266 nm

**Column:** 4.1-mm × 25-cm; packing L21

**Column temperature:** 35°

**Flow rate:** 1 mL/min

**Injection size:** 50 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Capacity factor:** NLT 2.0

**Relative standard deviation:** NMT 2.0% for replicate injections

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*

Calculate the percentage of the label claim in the portion of  $C_4H_{13}NO_7P_2$  taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of anhydrous USP Alendronate Sodium RS in the *Standard stock solution* (mg/mL)

$C_U$  = nominal concentration of alendronic acid in the *Sample stock solution* (mg/mL)

$M_{r1}$  = molecular weight of alendronic acid, 249.10

$M_{r2}$  = molecular weight of anhydrous alendronate sodium, 271.09

**Acceptance criteria:**  $C_4H_{12}NNaO_7P_2$  equivalent to 90.0%–110.0% of the labeled amount of  $C_4H_{13}NO_7P_2$

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

###### Test 1

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 15 min

Determine the amount of  $C_4H_{13}NO_7P_2$  dissolved by using the following method.

**Solution A and Mobile phase:** Proceed as directed in the *Assay*.

**Diluent:** 176.4 g/L of sodium citrate in *Medium*

**Solution B:** Dissolve 6.2 g of boric acid in approximately 950 mL of water. Adjust with 1 N sodium hydroxide to a pH of 9.0, and dilute with water to 1 L.

**Solution C:** 0.5 mg/mL of 9-fluorenylmethyl chloroformate in acetonitrile. [NOTE—Prepare this solution fresh.]

**Standard stock solution:** USP Alendronate Sodium RS in *Medium* to make a concentration equivalent to dissolving 1 Tablet in 900 mL of the same *Medium*. Calculate the concentration,  $C$  (mg/mL), of anhydrous alendronate sodium in this solution.

**Standard solution:** Transfer 5.0 mL of the *Standard stock solution* to a 50-mL polypropylene screw-cap centrifuge tube containing 1.0 mL of *Diluent* and 5.0 mL of *Solution B*, and mix for 3 min. Add 4.0 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 5 min. Use a portion of the clear, upper aqueous layer.



**Blank:** Transfer 5 mL of water to a 50-mL polypropylene screw-cap centrifuge tube containing 1.0 mL of *Diluent* and 5.0 mL of *Solution B*, and mix for 3 min. Add 4.0 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 5 min. Use a portion of the clear, upper aqueous layer.

**Sample solution:** Withdraw a portion of the solution under test, and centrifuge immediately. Transfer 5.0 mL of the supernatant to a 50-mL polypropylene screw-cap centrifuge tube containing 1.0 mL of *Diluent* and 5.0 mL of *Solution B*, and mix for 3 min. Add 4.0 mL of *Solution C*, and agitate for 30 s. Allow the solution to stand at room temperature for 25 min. Add 25 mL of methylene chloride, and agitate for 40 s. Centrifuge the mixture for 5 min. Use a portion of the clear, upper aqueous layer.

**Chromatographic system and System suitability:**

Proceed as directed in the *Assay*.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_4H_{13}NO_7P_2$  dissolved:

$$\text{Result} = (r_U/r_S) \times C \times (M_{r1}/M_{r2}) \times V \times (100/L)$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C$  = defined under the *Standard stock solution*

$M_{r1}$  = molecular weight of alendronic acid, 249.10

$M_{r2}$  = molecular weight of alendronate sodium, 271.09

$V$  = volume of the *Medium*, 900 mL

$L$  = Tablet label claim (mg)

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_4H_{13}NO_7P_2$  is dissolved; for tablets labeled for weekly dosing, NLT 75% (Q) of the labeled amount of  $C_4H_{13}NO_7P_2$  is dissolved.

**Test 2**

If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

Determine the amount of  $C_4H_{12}NNaO_7P_2 \cdot 3H_2O$  dissolved using the following method.

**Solution B and Solution C:** Proceed as directed in the *Assay*.

**0.6 M citrate buffer:** 176.4 g/L of sodium citrate dihydrate in water

**0.05 M buffer:** Transfer 14.7 g of sodium citrate dihydrate and 7.05 g of anhydrous dibasic sodium phosphate to a 1000-mL volumetric flask, dissolve in about 900 mL of water, adjust with phosphoric acid to a pH of 8.0, and dilute with water to volume.

**Mobile phase:** 0.05 M buffer, acetonitrile, and methanol (76:19:5)

**Standard stock solution:** Prepare a solution of USP Alendronate Sodium RS in *Medium* with a final concentration corresponding to the concentration obtained by dissolving 1 tablet in 900 mL of *Medium*. Calculate the concentration,  $C$  (mg/mL), of anhydrous alendronate sodium in this solution.

**Standard solution:** Transfer 5.0 mL of the *Standard stock solution* to a 50-mL screw-cap polypropylene centrifuge tube containing 1.0 mL of 0.6 M citrate buffer and 5.0 mL of *Solution B*, and mix for about 3 min. Add 4.0 mL of *Solution C*, and agitate for about 30 s. Allow the solution to stand at room temperature for about 30 min. Add 25 mL of methylene chloride, and agitate vigorously for about 40 s. Centrifuge the mixture for 10 min. Use a portion of the clear upper aqueous layer.

**Blank:** Using 5 mL of water, proceed as directed for the *Standard solution*, beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

**Sample solution**

**For Tablets labeled to contain 5 mg, 10 mg, 35 mg, or 40 mg:** After 30 min, withdraw 30 mL of the solution under test, and pass through a suitable 0.45- $\mu$ m filter, discarding the first 10 mL. Using 5.0 mL of the filtrate, proceed as directed for the *Standard solution*, beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

**For Tablets labeled to contain 70 mg:** After 30 min, withdraw 30 mL of the solution under test, and pass through a suitable 0.45- $\mu$ m filter, discarding the first 10 mL. Transfer 6.0 mL of the filtrate to a 10-mL volumetric flask, and dilute with water to volume. Using 5.0 mL of this dilution, proceed as directed for the *Standard solution*, beginning with "to a 50-mL screw-cap polypropylene centrifuge tube".

**Chromatographic system and System suitability:**

Proceed as directed in the *Assay*.

**Analysis:** Proceed as directed in *Test 1*.

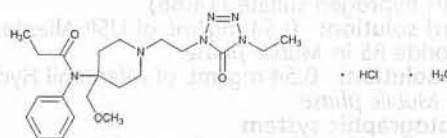
**Tolerances:** NLT 80% (Q) of the labeled amount of alendronate sodium ( $C_4H_{12}NNaO_7P_2 \cdot 3H_2O$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 15° and 30°.
- **LABELING:** The labeling indicates weekly dosing where appropriate. When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**  
USP Alendronate Sodium RS

## Alfentanil Hydrochloride



$C_{21}H_{32}N_6O_3 \cdot HCl \cdot H_2O$  470.99

$C_{21}H_{32}N_6O_3 \cdot HCl$  452.98

Propanamide, *N*-[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(methoxymethyl)-4-piperidinyl]-*N*-phenyl, monohydrochloride, monohydrate;

*N*-[1-[2-(4-Ethyl-5-oxo-2-tetrazolin-1-yl)-ethyl]-4-(methoxymethyl)-4-piperidyl]propionanilide monohydrochloride monohydrate [70879-28-6].

Anhydrous [69049-06-5].

**DEFINITION**

Alfentanil Hydrochloride contains NLT 98.0% and NMT 102.0% of alfentanil hydrochloride ( $C_{21}H_{32}N_6O_3 \cdot HCl$ ), calculated on the anhydrous basis.

**[CAUTION—**Handle Alfentanil Hydrochloride with great care because it is a potent opioid analgesic. Great care should be taken to prevent inhaling particles of Alfentanil Hydrochloride and exposing the skin to it.]

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.



**ASSAY**• **PROCEDURE**

**Mobile phase:** Acetonitrile and 0.01 M tetrabutylammonium hydrogen sulfate (14:86)

**Standard solution:** 0.54 mg/mL of USP Alfentanil Hydrochloride RS in *Mobile phase*

**Sample solution:** 0.54 mg/mL of Alfentanil Hydrochloride in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.0-mm × 25-cm; 5-μm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 25 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 5400 theoretical plates

**Tailing factor:** NMT 1.3

**Relative standard deviation:** NMT 0.73%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of alfentanil hydrochloride ( $C_{21}H_{32}N_6O_3 \cdot HCl$ ) in the portion of Alfentanil Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Alfentanil Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Alfentanil Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES**

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **ORGANIC IMPURITIES**

**Mobile phase:** Acetonitrile and 0.01 M tetrabutylammonium hydrogen sulfate (14:86)

**Standard solution:** 0.54 mg/mL of USP Alfentanil Hydrochloride RS in *Mobile phase*

**Sample solution:** 0.54 mg/mL of Alfentanil Hydrochloride in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 25 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 5400 theoretical plates

**Tailing factor:** NMT 1.3

**Relative standard deviation:** NMT 0.73%

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Alfentanil Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of all the peak responses

**Acceptance criteria**

**Any single impurity:** NMT 0.5%

**Total impurities:** NMT 1.0%

**SPECIFIC TESTS**

• **WATER DETERMINATION, Method I** (921): NMT 4.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Alfentanil Hydrochloride RS

**Alfentanil Injection****DEFINITION**

Alfentanil Injection is a sterile solution of Alfentanil Hydrochloride in Water for Injection. It contains an amount of Alfentanil Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of alfentanil ( $C_{21}H_{32}N_6O_3$ ).

[**CAUTION**—Handle Alfentanil Injection with great care because it is a potent opioid analgesic.]

**IDENTIFICATION**

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Standard solution:** 0.54 mg/mL of USP Alfentanil Hydrochloride RS

**Sample solution:** 0.5 mg/mL of alfentanil in water

**Chromatographic system**

**Application volume:** 200 μL

**Developing solvent system:** Chloroform, methanol, and formic acid (85:10:5)

**Visualizing agent:** Dragendorff's reagent

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in the chapter.

**Acceptance criteria:** Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Acetonitrile and 0.01 M tetrabutylammonium hydrogen sulfate (14:86)

**Standard solution:** 0.54 mg/mL of USP Alfentanil Hydrochloride RS in saline TS

**Sample solution:** Equivalent to 0.50 mg/mL of alfentanil from a suitable volume of Injection in saline TS

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 25 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 5400 theoretical plates

**Tailing factor:** NMT 1.3

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of alfentanil ( $C_{21}H_{32}N_6O_3$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*



- $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Alfentanil Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of alfentanil in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of alfentanil, 416.52  
 $M_{r2}$  = molecular weight of alfentanil hydrochloride, 452.98

Acceptance criteria: 90.0%–110.0%

## IMPURITIES

### • ORGANIC IMPURITIES

**Mobile phase:** Acetonitrile and 0.01 M tetrabutylammonium hydrogen sulfate (14:86)

**Standard solution:** 0.54 mg/mL of USP Alfentanil Hydrochloride RS in saline TS

**Sample solution:** Equivalent to 0.50 mg/mL of alfentanil from a suitable volume of Injection in saline TS

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 25 μL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Column efficiency:** NLT 5400 theoretical plates

**Tailing factor:** NMT 1.3

**Relative standard deviation:** NMT 1.0%

### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_u/r_T) \times 100$$

$r_u$  = peak response of each impurity

$r_T$  = sum of all of the peaks

**Acceptance criteria:** The sum of all impurities is NMT 2.0%.

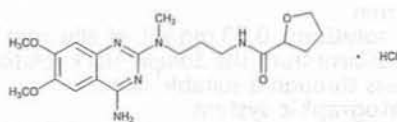
## SPECIFIC TESTS

- **pH (791):** 4.0–6.0
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 10 USP Endotoxin Units/mL
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, single-dose or multiple-dose containers, preferably of Type I glass, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
 USP Alfentanil Hydrochloride RS  
 USP Endotoxin RS

## Alfuzosin Hydrochloride



$C_{19}H_{27}N_5O_4 \cdot HCl$  425.91  
 2-Furancarboxamide, N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl)methylamino]propyl]tetrahydro-, monohydrochloride (±);

(±)-N-[3-[(4-Amino-6,7-dimethoxy-2-quinazolinyl)methylamino]propyl]tetrahydro-2-furamide monohydrochloride [81403-68-1].

## DEFINITION

Alfuzosin Hydrochloride contains NLT 98.0% and NMT 102.0% of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ), calculated on the anhydrous and solvent-free basis.

## IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements
- **C.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

## ASSAY

### • PROCEDURE

[NOTE—Use low-actinic glassware.]

**Solution A:** 2 M sodium hydroxide

**Solution B:** 5.0 mL of perchloric acid in 900 mL of water. Adjust with *Solution A* to a pH of 3.5, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile and *Solution B* (30:70)

**Diluent:** Methanol and *Solution A* (500:3)

**Standard stock solution:** 0.5 mg/mL of USP Alfuzosin Hydrochloride RS in *Diluent*

**Standard solution:** 0.04 mg/mL of USP Alfuzosin Hydrochloride RS in *Mobile phase* from the *Standard stock solution*; the solution is stable for 14 h at 5°.

**Sample stock solution:** 0.5 mg/mL of Alfuzosin Hydrochloride in *Diluent*

**Sample solution:** 0.04 mg/mL of Alfuzosin Hydrochloride in *Mobile phase* from the *Sample stock solution*; the solution is stable for 14 h at 5°.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Column temperature:** 30°

**Autosampler temperature:** 5°

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 μL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Relative standard deviation:** NMT 1.0%

**Tailing factor:** NMT 1.5

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) in the portion of Alfuzosin Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Alfuzosin Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis

## IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

### • ORGANIC IMPURITIES

**Buffer:** Dilute 5 mL of perchloric acid in 900 mL of water. Adjust with 2 M sodium hydroxide to a pH of 3.5, and dilute with water to 1 L.

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Buffer* (20:1:80)

**System suitability solution:** 0.4 mg/mL of USP Alfuzosin System Suitability Mixture RS in *Mobile phase*



**Sample solution:** 0.40 mg/mL of Alfuzosin Hydrochloride in *Mobile phase*

**Reference solution:** 0.40 µg/mL of Alfuzosin Hydrochloride in *Mobile phase* from the *Sample solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Peak-to-valley ratio:** The ratio of the height of the furamide analog peak to the height of the valley between the furamide analog peak and alfuzosin is NLT 5.

#### Analysis

**Samples:** *Sample solution* and *Reference solution*

Calculate the percentage of each impurity in the portion of Alfuzosin Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (1/D) \times 100$$

$r_u$  = peak response of each impurity from the *Sample solution*

$r_s$  = peak response of alfuzosin from the *Reference solution*

$D$  = dilution factor between the *Sample solution* and the *Reference solution*, 1000

**Acceptance criteria:** See *Table 1*. [NOTE—Disregard any peak with an area less than 0.05%.]

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deacylatedalfuzosin <sup>a</sup>	0.5	0.20
Alfuzosin	1.0	—
Furamide analog <sup>b</sup>	1.2	— <sup>c</sup>
Any other individual, unidentified impurity	—	0.10
Total impurities	—	0.30

<sup>a</sup> N<sup>2</sup>-(3-Aminopropyl)-6,7-dimethoxy-N<sup>2</sup>-methylquinazoline-2,4-diamine.

<sup>b</sup> N-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl]furan-2-carboxamide.

<sup>c</sup> Furamide analog, a component of USP Alfuzosin System Suitability Mixture RS, is not a specified impurity.

#### SPECIFIC TESTS

##### • OPTICAL ROTATION (781)

**Sample solution:** 20 mg/mL in carbon dioxide-free water

**Acceptance criteria:** −0.10° to +0.10°

##### • WATER DETERMINATION, Method I (921): NMT 2.0%

##### • PH (791)

**Sample solution:** 20 mg/mL in carbon dioxide-free water

**Acceptance criteria:** 4.0–5.5

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light and humidity, and store at room temperature.

##### • USP REFERENCE STANDARDS (11)

USP Alfuzosin Hydrochloride RS

USP Alfuzosin System Suitability Mixture RS

Alfuzosin Hydrochloride containing approximately 0.4% furamide analog (N-[3-[(4-amino-6,7-dimethoxy-

quinazolin-2-yl)(methyl)amino]propyl]furan-2-carboxamide), and about 0.4% deacylated alfuzosin (N<sup>2</sup>-(3-aminopropyl)-6,7-dimethoxy-N<sup>2</sup>-methylquinazoline-2,4-diamine).

## Alfuzosin Hydrochloride Extended-Release Tablets

#### DEFINITION

Alfuzosin Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alfuzosin hydrochloride (C<sub>19</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub> · HCl).

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197):** [NOTE—Methods described in (197K) or (197A) may be used.]

**Sample:** Grind 4 Tablets, and add 20 mL of water.

[NOTE—When analyzing multi-layer Tablets, isolate the layer containing alfuzosin hydrochloride using a suitable tool.] Add 20 mL of strong ammonia solution. Extract with 20 mL of methylene chloride, and separate the organic layer. Repeat the extraction successively with 20 mL, then with 10 mL of methylene chloride. Wash the combined organic layers with 20 mL of water. Dry the organic solution using a phase separation filter. Take 2.0 mL of the dried organic solution, and mix with 200 mg of finely ground potassium bromide. Evaporate the methylene chloride at 60°, then at 105° for 30 min. Make a disk. Alternatively, evaporate methylene chloride from the dried organic solution at 60°, then at 105° for 30 min. Perform the IR spectrum.

**Acceptance criteria:** The maxima of the spectrum obtained from the *Sample* correspond in position and relative intensity to those obtained from USP Alfuzosin Hydrochloride RS, treated in the same manner as the *Sample*, beginning with "add 20 mL of water."

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Solution A:** 5.0 mL of perchloric acid in 900 mL of water. Adjust with 2 M sodium hydroxide to a pH of 3.5, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Solution A* (20:1:80)

**Diluent:** 0.01 N hydrochloric acid

**Standard stock solution:** 0.15 mg/mL of USP Alfuzosin Hydrochloride RS in methanol

**Standard solution:** 0.03 mg/mL of USP Alfuzosin Hydrochloride RS in *Diluent* from the *Standard stock solution*

**Sample stock solution:** Place a suitable number of Tablets into a suitable volumetric flask to obtain a solution having a concentration of 0.16 mg/mL of alfuzosin hydrochloride. Add 80% of the flask volume of methanol, and stir for at least 1 h using a magnetic stirrer. Add 10% of the flask volume of *Diluent*, mix, and allow it to cool to room temperature. Dilute the resulting suspension with methanol to volume, stir, and allow to settle for 30 min.

**Sample solution:** 0.03 mg/mL of alfuzosin hydrochloride in *Diluent* from the *Sample stock solution* supernatant. Pass through a suitable filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 254 nm  
 Column: 4.6-mm × 15-cm; 5-μm packing L1  
 Flow rate: 1.5 mL/min  
 Injection volume: 20 μL  
 System suitability  
 Sample: *Standard solution*  
 Suitability requirements  
 Tailing factor: 0.8–1.5 for alfuzosin  
 Relative standard deviation: NMT 2.0%  
 Analysis  
 Samples: *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### Change to read:

#### • DISSOLUTION (711)

##### Test 1

Medium: 0.01 N hydrochloric acid; 500 mL

Apparatus 2: 100 rpm, with Tablet holder (see Figure 1)

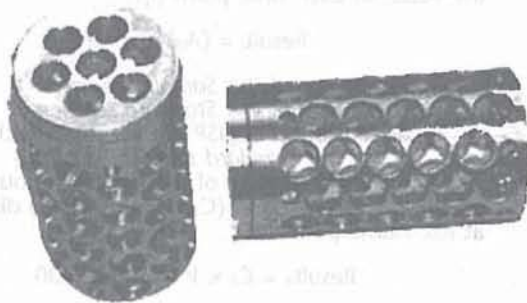


Figure 1. 37.5-mm (l) × 20-mm (d) stainless steel cylinders are used as sample holders. The cylinders contain screw caps drilled with seven 4.5-mm holes. Seven 4.5-mm holes are drilled in the bottom, and 12 longitudinal series of five 5-mm holes are drilled on the cylinders, alternatively starting and ending with one 1.7-mm hole.

Times: 1, 6, 12, and 20 h

Sample solution: Pass a portion of the solution under test through a suitable filter.

Standard solution: ( $L/500$ ) mg/mL of USP Alfuzosin Hydrochloride RS in *Medium*, where  $L$  is the Tablet label claim in mg

Detector: UV 330 nm

Blank: *Medium*

Path length: 1 cm

Tolerances: See Table 1.

Table 1

Level	Time (h)	Amount Dissolved (%)
L1		Each Tablet:
	1	10–20
	6	40–55
	12	65–85
	20	NLT 85
L2		Average of 12 Tablets complies with L1 and each Tablet:
	1	9–22
	6	36–61
	12	59–94
	20	NLT 77
L3		Average of 24 Tablets complies with L1, NMT 2 Tablets outside L2, and all Tablets within:
	1	8–24
	6	32–66
	12	52–102
	20	NLT 68

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 100 rpm

Times: 1, 3, 12, and 24 h

Buffer: Dilute 5.0 mL of perchloric acid in 900 mL of water, adjust with diluted sodium hydroxide (0.1 g/mL) to a pH of  $3.5 \pm 0.5$ , and dilute with water to 1 L.

Mobile phase: Acetonitrile and Buffer (25:75)

Standard stock solution: 0.28 mg/mL of USP Alfuzosin Hydrochloride RS, prepared as follows. In a 200-mL volumetric flask dissolve 55.5 mg of USP Alfuzosin Hydrochloride RS in 5 mL of methanol, sonicate to dissolve, and dilute with *Medium* to volume.

Standard solution: 0.011 mg/mL of USP Alfuzosin Hydrochloride RS in *Medium* from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter. Replace the portion of solution withdrawn with an equal volume of *Medium*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 244 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection volume: 20 μL

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 3000 theoretical plates

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration ( $C$ ) of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result}_i = (r_U/r_S) \times C_S$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)



Calculate the percentage of the labeled amount of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$C_i$  = concentration of alfuzosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)  
 $V$  = volume of *Medium*, 900 mL  
 $L$  = label claim (mg/Tablet)  
 $V_3$  = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See Table 2.

Table 2

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 20
2	3	15–35
3	12	50–70
4	24	NLT 80

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium:** 0.25% sodium dodecyl sulfate in 0.05 M sodium phosphate buffer, pH 6.8 (2.5 g/L of sodium dodecyl sulfate, 6.9 g/L of monobasic sodium phosphate monohydrate, and 0.83 g/L of sodium hydroxide in water previously degassed with helium. Adjust with either phosphoric acid or 1 N sodium hydroxide to a pH of  $6.8 \pm 0.05$ ); 900 mL

**Apparatus 1:** 100 rpm

**Times:** 1, 6, 12, and 24 h

**Standard stock solution:** 1.1 mg/mL of USP Alfuzosin Hydrochloride RS in methanol

**Standard solution:** 0.011 mg/mL of USP Alfuzosin Hydrochloride RS in *Medium* from the *Standard stock solution*

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 331 nm, background correction at 490 nm

**Blank:** *Medium*

**Cell:** 1.0 cm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium* (mL)

$L$  = label claim (mg/Tablet)

**Tolerances:** See Table 3.

Table 3

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 15
2	6	20–40
3	12	45–70
4	24	NLT 80

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Medium:** 0.01 N hydrochloric acid; 900 mL

**Apparatus 2:** 100 rpm

**Times:** 1, 6, 12, and 20 h

**Standard solution:** 0.01 mg/mL of USP Alfuzosin Hydrochloride RS in *Medium*

**Sample solution:** Centrifuge a portion of the solution under test.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV

**Analytical wavelength:** 245 nm

**Blank:** *Medium*

**Path length:** 0.2 cm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration ( $C_i$ ) of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result}_1 = (A_U/A_S) \times C_S$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times (V - V_3)) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times [V - (2 \times V_3)]) + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times [V - (3 \times V_3)]) + [(C_3 + C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$C_i$  = concentration of alfuzosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

$V_3$  = volume of the *Sample solution* withdrawn at each time point (mL)

**Tolerances:** See Table 4.



Table 4

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 30
2	6	40–60
3	12	65–85
4	20	NLT 80

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

**Test 5:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

**Medium:** 0.01 N hydrochloric acid; 900 mL

**Apparatus 2:** 100 rpm

**Times:** 1, 3, 6, 12, and 20 h

**Buffer:** Add 1 mL of triethylamine in 1000 mL of water. Adjust with phosphoric acid to a pH of  $2.5 \pm 0.05$ . Pass through a suitable filter of 0.45- $\mu$ m pore size.

**Mobile phase:** Methanol and Buffer (40:60)

**Standard stock solution:** 0.55 mg/mL of USP Alfuzosin Hydrochloride RS. Prepare by transferring a portion of USP Alfuzosin Hydrochloride RS to a suitable flask. Add methanol to 20% of the flask volume, and sonicate at room temperature to dissolve. Dilute with Medium to volume.

**Standard solution:** 0.011 mg/mL of USP Alfuzosin Hydrochloride RS in Medium from the Standard stock solution. Pass through a suitable filter of 0.45- $\mu$ m pore size.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 245 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** Standard solution

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Column efficiency:** NLT 2000 theoretical plates

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the concentration ( $C_i$ ) of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result}_i = (r_u/r_s) \times C_s$$

$r_u$  = peak response from the Sample solution

$r_s$  = peak response from the Standard solution

$C_s$  = concentration of USP Alfuzosin Hydrochloride RS in the Standard solution (mg/mL)

Calculate the percentage of the labeled amount of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_s)] + (C_1 \times V_s)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_s)]] + [(C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times [V - (3 \times V_s)]] + [(C_3 + C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_5 = \{[C_5 \times [V - (4 \times V_s)]] + [(C_4 + C_3 + C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$C_i$  = concentration of alfuzosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)

$V$  = volume of Medium, 900 mL

$L$  = label claim (mg/Tablet)

$V_s$  = volume of the Sample solution withdrawn at each time point (mL)

**Tolerances:** See Table 5.

Table 5

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 20
2	3	20–40
3	6	35–55
4	12	60–80
5	20	NLT 80

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

**Test 6:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

**Medium:** 0.01 N hydrochloric acid; 900 mL

**Apparatus 2:** 100 rpm. Adjust the paddle height to 4.5 cm above the bottom of the vessel and use a 10# mesh basket as sinker.

**Times:** 1, 6, 12, and 20 h

**Buffer:** 2.3 g/L of anhydrous dibasic sodium phosphate and 1.75 g/L of monobasic potassium phosphate

**Mobile phase:** Acetonitrile and Buffer (50:50)

**Standard stock solution:** 0.45 mg/mL of USP Alfuzosin Hydrochloride RS. Prepare by transferring a portion of USP Alfuzosin Hydrochloride RS to a suitable flask. Add 20% of the flask volume of water. Sonicate to dissolve, and dilute with Medium to volume.

**Standard solution:** 0.011 mg/mL of USP Alfuzosin Hydrochloride RS in Medium from the Standard stock solution

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Replace with the same volume of Medium.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 254 nm  
 Column: 4.6-mm × 25-cm; 5-μm packing L1  
 Column temperature: 30°  
 Flow rate: 1 mL/min  
 Injection volume: 20 μL

**System suitability**

Sample: *Standard solution*  
 Suitability requirements  
 Tailing factor: NMT 2.0  
 Column efficiency: NLT 3500 theoretical plates  
 Relative standard deviation: NMT 2.0%

**Analysis**

Samples: *Standard solution* and *Sample solution*  
 Calculate the concentration ( $C_i$ ) of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result}_i = (r_u/r_s) \times C_s$$

$r_u$  = peak response from the *Sample solution*  
 $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_s)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$C_i$  = concentration of alfuzosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

$V_s$  = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See Table 6.

**Table 6**

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 30
2	6	45–65
3	12	70–90
4	20	NLT 85

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

• **Test 7:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 7*.  
 Medium: 0.01 N hydrochloric acid; 900 mL  
 Apparatus 2: 100 rpm with sinker; see *Dissolution* (711), *Figure 2a*.

Times: 1, 6, 12, and 20 h

Solution A: 500 g/L of sodium hydroxide

Solution B: 100 g/L of sodium hydroxide

Buffer: Add 5 mL of perchloric acid in 1000 mL of water. Adjust with *Solution A* to a pH of 2.5, then adjust with *Solution B* to a pH of  $3.50 \pm 0.05$ . Pass through a suitable filter of 0.45-μm pore size.

Mobile phase: Acetonitrile and *Buffer* (24:76)

Standard solution: 0.01 mg/mL of USP Alfuzosin Hydrochloride RS in *Medium*

Sample solution: Centrifuge or pass a portion of the solution under test through a suitable filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 245 nm

Column: 4.6-mm × 5-cm; 3-μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 10 μL

Run time: NLT 1.5 times the retention time of alfuzosin

**System suitability**

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.8

Relative standard deviation: NMT 2.0%

**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the concentration ( $C_i$ ) of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result}_i = (r_u/r_s) \times C_s$$

$r_u$  = peak response from the *Sample solution*  
 $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alfuzosin hydrochloride ( $C_{19}H_{27}N_5O_4 \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times (V - V_s)) + (C_1 \times V_s)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times [V - (2 \times V_s)]) + [(C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times [V - (3 \times V_s)]) + [(C_3 + C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$C_i$  = concentration of alfuzosin hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

$V_s$  = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See Table 7.

**Table 7**

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 25
2	6	40–60
3	12	65–85
4	20	NLT 85

The percentages of the labeled amount of alfuzosin hydrochloride dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*. (RB 1-Jun-2016)

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements



## IMPURITIES

## Change to read:

## • ORGANIC IMPURITIES

**Solution A, Mobile phase, Diluent, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**System suitability stock solution:** 0.4 mg/mL of USP Alfuzosin System Suitability Mixture A RS in methanol

**System suitability solution:** 0.03 mg/mL of USP Alfuzosin System Suitability Mixture A RS in *Diluent* from the *System suitability stock solution*

**Standard stock solution:** 0.15 mg/mL of USP Alfuzosin Hydrochloride RS in methanol

**Standard solution:** 0.03 mg/mL of USP Alfuzosin Hydrochloride RS in *Diluent* from the *Standard stock solution*

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.0 between alfuzosin and the furamide analog; NLT 1.0 between deacylated alfuzosin and the *N*-formyl analog, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of each impurity from the *Sample solution*  
 $r_s$  = peak response of alfuzosin from the *Sample solution*  
 $C_s$  = concentration of USP Alfuzosin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of alfuzosin hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See <sup>o</sup>Table 8.

Table 8<sup>o</sup> (RB 1-Jun-2016)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deacylated alfuzosin <sup>a</sup>	0.46	0.40
<i>N</i> -Formyl analog <sup>b</sup>	0.50	0.30
Alfuzosin	1.0	—
Furamide analog <sup>c</sup>	1.18	— <sup>d</sup>
Any individual unspecified impurity	—	0.20
Total impurities	—	0.80

<sup>a</sup> *N*-(3-Aminopropyl)-6,7-dimethoxy-*N*²-methylquinazoline-2,4-diamine.

<sup>b</sup> *N*-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl]formamide.

<sup>c</sup> *N*-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl]furan-2-carboxamide.

<sup>d</sup> Furamide analog, a component of USP Alfuzosin System Suitability Mixture A RS, is not a specified impurity.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Protect from light and moisture. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

## • USP REFERENCE STANDARDS (11)

USP Alfuzosin Hydrochloride RS

USP Alfuzosin System Suitability Mixture A RS

Furamide analog: *N*-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl]furan-2-carboxamide.

$C_{19}H_{23}N_5O_4$  385.42

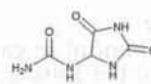
Deacylated alfuzosin: *N*²-(3-Aminopropyl)-6,7-dimethoxy-*N*²-methylquinazoline-2,4-diamine.

$C_{14}H_{21}N_5O_2$  291.35

*N*-Formyl analog: *N*-[3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl)(methyl)amino]propyl]formamide.

$C_{15}H_{21}N_5O_3$  319.36

## Allantoin



$C_4H_6N_4O_3$

158.12

Urea, (2,5-dioxo-4-imidazolidinyl)-;  
Allantoin [97-59-6].

## DEFINITION

Allantoin contains NLT 98.5% and NMT 101.0% of  $C_4H_6N_4O_3$ .

## IDENTIFICATION

## • A. INFRARED ABSORPTION (197K)

## • B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST

(201): The  $R_f$  value of the principal spot from *Sample solution B* corresponds to that from *Standard solution A*, as described in the test for *Organic Impurities*.

## ASSAY

## • PROCEDURE

**Sample:** 120 mg

**Analysis:** Transfer the *Sample* to a 100-mL beaker, dissolve by stirring in 40 mL of water, and titrate with 0.1 M sodium hydroxide. Use a suitable electrode system (see *Titrimetry* (541)). Each mL of 0.1 M sodium hydroxide is equivalent to 15.81 mg of  $C_4H_6N_4O_3$ .

Acceptance criteria: 98.5%–101.0%

## IMPURITIES

## • RESIDUE ON IGNITION (281): NMT 0.1%

## • ORGANIC IMPURITIES

**Adsorbent:** Cellulose

**Diluent:** Methanol and water (1:1)

**Urea stock solution:** 1 mg/mL of USP Urea RS in water

**Standard solution A:** 1 mg/mL of USP Allantoin RS in *Diluent*

**Standard solution B:** 0.1 mg/mL of USP Urea RS in methanol, from *Urea stock solution*

**Standard solution C:** *Standard solution A* and *Standard solution B* (1:1)

**Sample solution A:** Transfer 0.10 g of Allantoin to a 10-mL volumetric flask, add 5 mL of water, dissolve by heating, and allow to cool. Dilute with methanol to volume. [NOTE—Use immediately after preparation.]

**Sample solution B:** Transfer 1 mL of *Sample solution A* to a 10-mL volumetric flask, and dilute with *Diluent* to volume.

**Spray reagent:** 5 mg/mL of *p*-dimethylaminobenzaldehyde in a mixture of methanol and hydrochloric acid (3:1)



**Application volume**Standard solution A: 5  $\mu$ LStandard solution B: 5  $\mu$ LStandard solution C: 5  $\mu$ LSample solution A: 10  $\mu$ LSample solution B: 5  $\mu$ L**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (60:15:25)**Analysis:** Proceed as directed for *Chromatography* (621), *Thin-Layer Chromatography*. Develop the chromatogram until the solvent front has moved about 10 cm. Spray the plate with *Spray reagent*, dry in a current of hot air, and after 30 min examine under visible light.**Acceptance criteria:** Any spot from *Sample solution A*, except for the principal spot, is not more intense than the spot from *Standard solution B* (NMT 0.5%). The test is not valid unless the principal spots from *Standard solution C* are clearly separated.**SPECIFIC TESTS****• ACIDITY OR ALKALINITY****Sample solution:** 5 mg/mL in carbon dioxide-free water  
**Analysis:** To 5 mL of the *Sample solution* add 5 mL of water, 0.1 mL of methyl red TS, and 0.2 mL of 0.01 M sodium hydroxide.**Acceptance criteria:** A yellow color is observed. The solution turns red upon the addition of 0.4 mL of 0.01 M hydrochloric acid.**• LOSS ON DRYING (731):** Dry a sample at 105° to constant weight: it loses NMT 0.1% of its weight.**• REDUCING SUBSTANCES****Sample solution:** 1.0 g of Allantoin in 10 mL of water. Shake for 2 min, and filter.**Analysis:** To the *Sample solution* add 1.5 mL of 0.02 M potassium permanganate.**Acceptance criteria:** The solution remains violet for at least 10 min.**ADDITIONAL REQUIREMENTS****• USP REFERENCE STANDARDS (11)**

USP Allantoin RS

USP Urea RS

**Allopurinol** $C_5H_4N_4O$ 

136.11

4*H*-Pyrzolo[3,4-*d*]pyrimidin-4-one, 1,5-dihydro-;  
1,5-Dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one;  
1*H*-Pyrzolo[3,4-*d*]pyrimidin-4-ol [315-30-0].**DEFINITION**Allopurinol contains NLT 98.0% and NMT 102.0% of allopurinol ( $C_5H_4N_4O$ ), calculated on the dried basis.**IDENTIFICATION****• INFRARED ABSORPTION (197K)****ASSAY****• PROCEDURE**[NOTE—Store and inject the *System suitability solution*, *Standard solution*, and *Sample solution* at 8°, using a cooled autosampler.]**Mobile phase:** 1.25-g/L solution of monobasic potassium phosphate in water, filtered and degassed**System suitability solution:** 0.5  $\mu$ g/mL each of USP Allopurinol RS, USP Allopurinol Related Compound B RS, and USP Allopurinol Related Compound C RS, preparedas follows. Transfer weighed quantities of USP Allopurinol RS, USP Allopurinol Related Compound B RS, and USP Allopurinol Related Compound C RS to three separate suitable volumetric flasks, dissolve in a small volume of 0.1 N sodium hydroxide, and immediately dilute with *Mobile phase* to volume to obtain solutions containing 0.05 mg/mL each. Transfer 1.0 mL of each of these three solutions to a 100-mL volumetric flask and dilute with *Mobile phase* to volume.**Standard stock solution:** 0.5 mg/mL of USP Allopurinol RS, prepared as follows. Transfer a weighed quantity of USP Allopurinol RS to a suitable volumetric flask, dissolve in a small volume of 0.1 N sodium hydroxide, and immediately dilute with *Mobile phase* to volume.**Standard solution:** 0.08 mg/mL of USP Allopurinol RS in *Mobile phase* from the *Standard stock solution***Sample stock solution:** 0.5 mg/mL of Allopurinol, prepared as follows. Transfer 50 mg of Allopurinol to a 100-mL volumetric flask, dissolve in 5.0 mL of 0.1 N sodium hydroxide, and immediately dilute with *Mobile phase* to volume.**Sample solution:** 0.08 mg/mL of Allopurinol in *Mobile phase* from the *Sample stock solution***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column:** 4.6-mm  $\times$  25-cm; packing L1**Flow rate:** 1.8 mL/min**Injection volume:** 20  $\mu$ L**System suitability****Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for allopurinol related compound B, allopurinol related compound C, and allopurinol are about 0.7, 0.8, and 1.0, respectively.]

**Suitability requirements****Resolution:** NLT 1.1 between allopurinol related compound B and allopurinol related compound C; NLT 6.0 between allopurinol related compound C and allopurinol, *System suitability solution***Relative standard deviation:** NMT 2.0% for replicate injections, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of allopurinol ( $C_5H_4N_4O$ ) in the portion of Allopurinol taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 $r_u$  = peak response from the *Sample solution* $r_s$  = peak response from the *Standard solution* $C_s$  = concentration of USP Allopurinol RS in the *Standard solution* (mg/mL) $C_u$  = concentration of Allopurinol in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the dried basis**IMPURITIES****• ORGANIC IMPURITIES**[NOTE—Store and inject the *Standard solution* and the *Sample solution* at 8°, using a cooled autosampler.]**Solution A:** 1.25-g/L solution of monobasic potassium phosphate in water, filtered and degassed**Solution B:** Methanol**Mobile phase:** See Table 1.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30	70	30
35	70	30



Table 1 (Continued)

Time (min)	Solution A (%)	Solution B (%)
36	90	10
46	90	10

**Diluent:** Solution A and Solution B (90:10)

**Standard stock solution:** 0.05 mg/mL each of USP Allopurinol RS, USP Allopurinol Related Compound A RS, USP Allopurinol Related Compound B RS, USP Allopurinol Related Compound C RS, USP Allopurinol Related Compound D RS, and USP Allopurinol Related Compound E RS, prepared as follows. Transfer 5 mg each of USP Allopurinol RS, USP Allopurinol Related Compound A RS, USP Allopurinol Related Compound B RS, USP Allopurinol Related Compound C RS, USP Allopurinol Related Compound D RS, and USP Allopurinol Related Compound E RS to a 100-mL volumetric flask. Add 2.0 mL of 0.1 N sodium hydroxide, and promptly sonicate with swirling for NMT 1 min to dissolve. Add 80 mL of *Diluent*, and sonicate for an additional 5 min. Dilute with *Diluent* to volume. [NOTE—This solution is stable for 48 h when stored at 8°.]

**Standard solution:** 0.5 µg/mL each of USP Allopurinol RS, USP Allopurinol Related Compound A RS, USP Allopurinol Related Compound B RS, USP Allopurinol Related Compound C RS, USP Allopurinol Related Compound D RS, and USP Allopurinol Related Compound E RS in *Diluent* from the *Standard stock solution*

**Sample solution:** 0.25 mg/mL of Allopurinol, prepared as follows. Transfer 25 mg of Allopurinol to a 100-mL volumetric flask. Add 5.0 mL of 0.1 N sodium hydroxide to dissolve, promptly sonicate with swirling for NMT 1 min, add 80 mL of *Diluent*, and sonicate for an additional 5 min. Dilute with *Diluent* to volume.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1.0 mL/min

**Injection volume:** 40 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—See Table 2 for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 0.8 between allopurinol related compound C and allopurinol related compound B

**Tailing factor:** NMT 1.5 for the allopurinol peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentages of allopurinol related compounds A, B, C, D, and E in the portion of Allopurinol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of each individual impurity from the *Standard solution*

$C_S$  = concentration of each individual impurity in the *Standard solution* (mg/mL)

$C_U$  = concentration of Allopurinol in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Allopurinol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of allopurinol from the *Standard solution*

$C_S$  = concentration of USP Allopurinol RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Allopurinol in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Allopurinol related compound A	0.62	0.2
Allopurinol related compound C	0.79	0.2
Allopurinol related compound B	0.81	0.2
Allopurinol	1.0	—
Allopurinol related compound D	4.4	0.2
Allopurinol related compound E	4.8	0.2
Ethyl-(E/Z)-3-(2-carboxy-2-cyanoethenyl)amino-1H-pyrazole-4-carboxylate	6.5	0.2
Unspecified impurity	—	0.1
Total impurities	—	1.0

#### • LIMIT OF HYDRAZINE

[NOTE—Under the following conditions, any hydrazine present in the sample will react with benzaldehyde to form benzalazine.]

**Mobile phase:** Hexane and isopropyl alcohol (95:5)

**2 N sodium hydroxide solution:** Dissolve 8.5 g of sodium hydroxide in water, and dilute with the same solvent to 100 mL. Alternatively, a commercially available 2 N sodium hydroxide solution can be used.

**Diluent:** Methanol and 2 N sodium hydroxide solution (1:1)

**Benzaldehyde solution:** 40 mg/mL of benzaldehyde in *Diluent*. [NOTE—Prepare immediately before use.]

**Hydrazine solution:** 2.0 µg/mL of hydrazine sulfate in *Diluent*. Use sonication if necessary.

**Standard solution:** Transfer 5.0 mL of *Hydrazine solution* to a suitable flask and add 4 mL of *Benzaldehyde solution*. Mix and allow to stand for 2.5 h at room temperature. Add 5.0 mL of hexane, and shake for 1 min. Allow the layers to separate, and use the upper (hexane) layer.

**Allopurinol solution:** Dissolve 250 mg of Allopurinol in 5 mL of *Diluent*.

**Sample solution:** Transfer the *Allopurinol solution* to a suitable flask, and add 4 mL of *Benzaldehyde solution*. Mix, and allow to stand for 2.5 h at room temperature. Add 5.0 mL of hexane, and shake for 1 min. Allow the layers to separate, and use the upper (hexane) layer.

**Blank solution:** Mix 5.0 mL of *Diluent* and 4 mL of *Benzaldehyde solution*, and allow to stand for 2.5 h at room temperature. Add 5.0 mL of hexane, and shake for 1 min. Allow the layers to separate, and use the upper (hexane) layer.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)



Mode: LC

Detector: UV 310 nm

Column: 4.0-mm × 25-cm; 5-μm packing L10

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

Sample: Standard solution

[NOTE—The relative retention times for benzalazine and benzaldehyde are about 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between benzalazine and benzaldehyde

Relative standard deviation: NMT 15.0% for the benzalazine peak

Analysis

Samples: Standard solution and Sample solution

Calculate the amount, in ppm, of hydrazine in the portion of Allopurinol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times F$$

 $r_U$  = peak response of benzalazine from the Sample solution

 $r_S$  = peak response of benzalazine from the Standard solution

 $C_S$  = concentration of hydrazine sulfate in the Hydrazine solution (μg/mL)

 $C_U$  = concentration of Allopurinol in the Allopurinol solution (mg/mL)

 $M_{r1}$  = molecular weight of hydrazine, 32.05

 $M_{r2}$  = molecular weight of hydrazine sulfate, 130.12

 $F$  = unit conversion factor (from μg/mg to ppm), 1000

Acceptance criteria: NMT 10 ppm of hydrazine

**SPECIFIC TESTS**• **LOSS ON DRYING** (731)

Analysis: Dry under vacuum at 105° for 5 h.

Acceptance criteria: NMT 0.5%

**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.• **USP REFERENCE STANDARDS** (11)

USP Allopurinol RS

USP Allopurinol Related Compound A RS

3-Amino-4-carboxamidopyrazole hemisulfate.

(C<sub>5</sub>H<sub>6</sub>N<sub>4</sub>O)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> 350.32

USP Allopurinol Related Compound B RS

5-(Formylamino)-1H-pyrazole-4-carboxamide.

C<sub>5</sub>H<sub>6</sub>N<sub>4</sub>O<sub>2</sub> 154.13

USP Allopurinol Related Compound C RS

5-(4H-1,2,4-Triazol-4-yl)-1H-pyrazole-4-carboxamide.

C<sub>6</sub>H<sub>6</sub>N<sub>6</sub>O 178.15

USP Allopurinol Related Compound D RS

Ethyl 5-amino-1H-pyrazole-4-carboxylate.

C<sub>6</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub> 155.15

USP Allopurinol Related Compound E RS

Ethyl 5-(formylamino)-1H-pyrazole-4-carboxylate.

C<sub>7</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub> 183.16

## Allopurinol Compounded Oral Suspension

**DEFINITION**

Allopurinol Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of allopurinol (C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O).

Prepare Allopurinol Compounded Oral Suspension 20 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Allopurinol tablets equivalent to	2 g of allopurinol
Glycerin	5 mL
Vehicle for Oral Suspension, <i>NF</i>	45 mL
Vehicle for Oral Solution, <i>NF</i> , a sufficient quantity to make	100 mL

Select the number of tablets that contain the specified amount of allopurinol, and calculate the quantity of each ingredient required for the total amount to be prepared. Count, weigh, or measure each ingredient. Thoroughly pulverize the tablets. Mix the powdered *Allopurinol tablets* and *Glycerin* to form a smooth paste. Incorporate the *Vehicle for Oral Suspension*. Add sufficient *Vehicle for Oral Solution* to volume, and mix well. Adjust the pH, if necessary. Package and label.

**SPECIFIC TESTS**• **pH** (791): 6.5–7.5**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Package in a tight container, and store at controlled room temperature.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature
- **LABELING:** Label it to state that it is to be shaken well before use, and to state the *Beyond-Use Date*.

## Allopurinol Tablets

» Allopurinol Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of allopurinol (C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Allopurinol RS

**Identification**—Extract a quantity of finely powdered Tablets, equivalent to about 50 mg of allopurinol, by trituration with 10 mL of 0.1 N sodium hydroxide. Filter, acidify the filtrate with 1 N acetic acid, collect the precipitated allopurinol (allow 10 to 15 minutes for sufficient precipitation to occur), wash the precipitate with 3 mL of dehydrated alcohol, in portions, and finally wash with 4 mL of anhydrous ethyl ether. Allow to dry in air for 15 minutes, then dry at 105° for 3 hours: the residue so obtained meets the requirements for the *Identification* test under *Allopurinol*.

**Dissolution** (711)—

Medium: 0.01 N hydrochloric acid; 900 mL.

Apparatus 2: 75 rpm.

Time: 45 minutes.

**Standard stock solution**—Prepare a stock solution by transferring about 40 mg of USP Allopurinol RS, accurately weighed, to a 200-mL volumetric flask. Add 10 mL of 0.1 N sodium hydroxide, sonicate for about 2 minutes, shake by mechanical means for about 10 minutes, dilute with *Dissolution Medium* to volume, and mix.

**Standard solution**—Dilute the *Standard stock solution* with *Dissolution Medium* to obtain a solution having a concentration similar to that expected in the solution under test.

**Procedure**—Determine the amount of C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O dissolved by employing UV absorption at the wavelength of maximum absorbance at about 250 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with the *Standard solution*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O is dissolved in 45 minutes.



**Uniformity of dosage units** (905): meet the requirements.

**Assay**—[NOTE—Do not allow the *Mobile phase* to remain in the column overnight. After performing the procedure, flush the system with water for not less than 20 minutes, and then flush with methanol for 20 minutes.]

**Mobile phase**—Prepare a filtered and degassed 0.05 M solution of monobasic ammonium phosphate.

**Internal standard solution**—On the day of use, dissolve about 50 mg of hypoxanthine in 10 mL of 0.1 N sodium hydroxide, shake by mechanical means until dissolved (about 10 minutes), dilute with water to 50 mL, and mix.

**Standard preparation**—On the day of use, transfer about 50 mg of USP Allopurinol RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of 0.1 N sodium hydroxide, shake by mechanical means for 10 minutes, dilute with water to volume, and mix. Transfer 4.0 mL of this solution and 2.0 mL of *Internal standard solution* to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of allopurinol, to a 50-mL volumetric flask, add 10 mL of 0.1 N sodium hydroxide, shake by mechanical means for 10 minutes, add water to volume, and mix. [NOTE—From this point, conduct the remainder of the Assay without delay.] Filter, rejecting the first 10 mL of the filtrate. Transfer 4.0 mL of the filtrate and 2.0 mL of *Internal standard solution* to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for hypoxanthine and 1.0 for allopurinol; the resolution,  $R_s$ , between the analyte and internal standard is not less than 5; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of allopurinol ( $C_5H_4N_4O$ ) in the portion of Tablets taken by the formula:

$$2.5C(R_U / R_S)$$

in which  $C$  is the concentration, in µg per mL, of USP Allopurinol RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of allopurinol to hypoxanthine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Allyl Isothiocyanate



$C_4H_5NS$

3-Isothiocyanato-1-propene;  
Isothiocyanic acid allyl ester [57-06-7].

99.15

### DEFINITION

Allyl Isothiocyanate contains NLT 93.0% and NMT 105.0% of allyl isothiocyanate ( $C_4H_5NS$ ).

[CAUTION—Allyl Isothiocyanate is a potent lachrymator, with a pungent, irritating odor. Care should be taken to protect the eyes, to prevent inhalation of fumes, and to avoid tasting.]

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197F): The spectrum exhibits pronounced peaks at about 700, 950, 980, 1300, 1340, 1350, 1410, 1420, 1650, 2100, and 2200  $cm^{-1}$ .

### ASSAY

#### PROCEDURE

**Sample solution**: Transfer 4 mL into a 100-mL volumetric flask, and dilute with alcohol to volume.

**Analysis**: Transfer 5.0 mL of the *Sample solution* to a 100-mL conical flask, and add 50.0 mL of 0.1 N silver nitrate VS and 5 mL of ammonia TS. Connect the flask to a reflux condenser, heat on a water bath for 1 h, and allow to cool to room temperature. Disconnect the flask from the condenser, transfer the contents of the conical flask to a 100-mL volumetric flask with the aid of water, and dilute with water to volume. Pass through a dry filter, discarding the first 10 mL of the filtrate. To 50.0 mL of the subsequent filtrate add 5 mL of nitric acid and 2 mL of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Perform a blank determination, using 5 mL of alcohol in place of the *Sample solution*, and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 4.958 mg of allyl isothiocyanate ( $C_4H_5NS$ ).

Acceptance criteria: 93.0%–105.0%

### IMPURITIES

#### LIMIT OF PHENOLS

**Sample solution**: Dilute a 1-mL sample with 5 mL of alcohol.

**Analysis**: Add 1 drop of ferric chloride TS to the *Sample solution*.

Acceptance criteria: A blue color is not produced immediately.

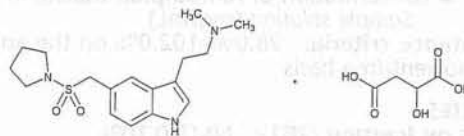
### SPECIFIC TESTS

- SPECIFIC GRAVITY** (841): 1.013–1.020
- REFRACTIVE INDEX** (831): 1.527–1.531, determined at 20°
- DISTILLING RANGE, Method I** (721): 148°–154°

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE**: Preserve in tight containers.

## Almotriptan Malate



$C_{17}H_{25}N_3O_2S \cdot C_4H_6O_5$

469.55

Pyrrolidine, 1-[[[3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]methyl]sulfonyl]-, hydroxybutanedioate (1:1); 1-[[[3-[2-(Dimethylamino)ethyl]indol-5-yl]methyl]sulfonyl]pyrrolidine malate (1:1) [181183-52-8].

### DEFINITION

Almotriptan Malate contains NLT 98.0% and NMT 102.0% of almotriptan malate ( $C_{17}H_{25}N_3O_2S \cdot C_4H_6O_5$ ), calculated on the anhydrous and solvent-free basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.



**ASSAY****• PROCEDURE**

**Buffer:** 2.72 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

**Mobile phase:** Methanol and Buffer (40:60)

**System suitability solution:** 0.14 mg/mL each of USP Almotriptan Malate RS and USP Almotriptan Related Compound B RS in *Mobile phase*. Sonication may be used to promote dissolution.

**Standard solution:** 0.14 mg/mL of USP Almotriptan Malate RS in *Mobile phase*. Sonication may be used to promote dissolution.

**Sample solution:** 0.14 mg/mL of Almotriptan Malate in *Mobile phase*. Sonication may be used to promote dissolution.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L10

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**Run time:** 2 times the retention time of almotriptan

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for almotriptan related compound B and almotriptan are 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between almotriptan and almotriptan related compound B, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 0.85% for six injections, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of almotriptan malate ( $C_{17}H_{25}N_3O_2S \cdot C_4H_6O_5$ ) in the portion of Almotriptan Malate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of almotriptan from the *Sample solution*

$r_S$  = peak response of almotriptan from the *Standard solution*

$C_S$  = concentration of USP Almotriptan Malate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Almotriptan Malate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis

**IMPURITIES**

**• RESIDUE ON IGNITION** (281): NMT 0.10%

**• LIMIT OF ALMOTRIPTAN RELATED COMPOUND D AND ALMOTRIPTAN N-DIMER**

**Run buffer:** 23.5 g/L of phosphoric acid in water. Adjust with triethanolamine to a pH of 3.0 and pass through a suitable filter of 0.45-μm pore size.

**Diluent:** Methanol and water (50:50)

**Internal standard solution:** 0.01 mg/mL of 4-hydroxy-4-phenylpiperidine in *Diluent*

**Standard stock solution:** 0.5 mg/mL of USP Almotriptan Malate RS in *Diluent*

**Standard solution:** 0.005 mg/mL of USP Almotriptan Malate RS from *Standard stock solution* in *Internal standard solution*. Pass through a suitable filter of 0.45-μm pore size.

**System suitability solution:** 0.005 mg/mL each of USP Almotriptan Related Compound B RS, USP Almotriptan Related Compound C RS, USP Almotriptan Related Compound D RS, and USP Almotriptan Malate RS in

*Internal standard solution*. Pass through a suitable filter of 0.45-μm pore size.

**Sample solution:** 2.5 mg/mL of Almotriptan Malate in *Internal standard solution*. Sonication may be used to promote dissolution. Pass the solution through a suitable filter of 0.45-μm pore size.

**Instrumental conditions**

**Mode:** CE

**Detector:** UV 214 nm

**Capillary dimensions:** 75-μm × 47-cm; uncoated fused silica

**Separation voltage:** 15 kV

**Electrokinetic injection:** 8s + 1s, *Run buffer*

**Run time:** NLT 2.5 times the migration time of almotriptan

Pre-condition the capillary by rinsing with water, 0.1 N sodium hydroxide solution, and *Run buffer* before each injection.

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 1 for the relative migration times.]

**Suitability requirements**

**Resolution:** NLT 2.0 between almotriptan related compound B and almotriptan; NLT 2.0 between almotriptan related C and almotriptan related compound D, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for the ratio of the peak response of almotriptan to the peak response of the internal standard, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the corrected peak response:

$$\text{Result} = (r/m)$$

$r$  = peak response

$m$  = migration time of the peak (min)

Calculate the percentage of almotriptan related compound D, almotriptan N-dimer, and other impurities in the portion of Almotriptan Malate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = corrected peak response ratio of the impurity to the internal standard from the *Sample solution*

$R_S$  = corrected peak response ratio of almotriptan to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Almotriptan Malate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Almotriptan Malate in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 1.

**Table 1**

Name	Relative Migration Time	Acceptance Criteria, NMT (%)
Almotriptan N-dimer <sup>a</sup>	0.71	0.3
Internal standard <sup>b</sup>	0.78	—
Almotriptan related compound B <sup>c</sup>	0.92	—
Almotriptan <sup>d</sup>	1.0	—
Almotriptan related compound C <sup>c,d</sup>	1.02	—

<sup>a</sup> 2-[(1-[(3-[2-(Dimethylamino)ethyl]-1H-indol-5-yl)methyl]-5-[(pyrrolidin-1-yl)sulfonyl)methyl]-1H-indol-3-yl)-N,N-dimethylethan-1-amine.

<sup>b</sup> 4-Hydroxy-4-phenylpiperidine.

<sup>c</sup> This impurity is quantified using the test for *Organic Impurities*.

<sup>d</sup> Almotriptan and almotriptan related compound C may not be fully resolved.



Table 1 (Continued)

Name	Relative Migration Time	Acceptance Criteria, NMT (%)
Almotriptan related compound D	1.22	0.1
Any individual unspecified impurities	—	0.1

<sup>a</sup> 2-[1-((3-[2-(Dimethylamino)ethyl]-1*H*-indol-5-yl)methyl)-5-[(pyrrolidin-1-ylsulfonyl)methyl]-1*H*-indol-3-yl)-*N,N*-dimethylethan-1-amine.

<sup>b</sup> 4-Hydroxy-4-phenylpiperidine.

<sup>c</sup> This impurity is quantified using the test for *Organic Impurities*.

<sup>d</sup> Almotriptan and almotriptan related compound C may not be fully resolved.

#### • ORGANIC IMPURITIES

**Buffer:** Add 10 mL of triethylamine to every 1000 mL of 0.01 M phosphoric acid. Adjust with phosphoric acid to a pH of 6.5.

**Mobile phase:** Acetonitrile and Buffer (15:85)

**System suitability stock solution:** 0.5 mg/mL each of USP Almotriptan Related Compound B RS, USP Almotriptan Related Compound C RS, and USP Almotriptan Related Compound D RS in methanol

**System suitability solution:** 0.005 mg/mL each of USP Almotriptan Related Compound B RS, USP Almotriptan Related Compound C RS, and USP Almotriptan Related Compound D RS from *System suitability stock solution* in water

**Standard solution:** 0.007 mg/mL of USP Almotriptan Malate RS in water

**Sample solution:** 3.5 mg/mL of Almotriptan Malate in water. Sonication may be used to promote dissolution.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 30-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 μL

**Run time:** 3 times the retention time of almotriptan

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.0 between almotriptan related compound C and almotriptan related compound D, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for six replicate injections, *Standard solution*

#### Analysis

**Samples:** *System suitability solution*, *Standard solution*, and *Sample solution*

Chromatograph the *System suitability solution* and identify the components on the basis of their relative retention times, as shown in Table 2.

Calculate the percentage of each impurity in the portion of Almotriptan Malate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of almotriptan from the *Standard solution*

$C_S$  = concentration of USP Almotriptan Malate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Almotriptan Malate in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Malic acid <sup>a</sup>	0.10	—
Almotriptan related compound B	0.62	0.1
Almotriptan related compound C	0.77	0.5
Almotriptan related compound D <sup>b</sup>	0.92	—
Almotriptan	1.00	—
Any other individual impurity	—	0.1
Total impurities <sup>c</sup>	—	0.7

<sup>a</sup> Included for identification purposes only.

<sup>b</sup> This impurity is quantified using the *Limit of Almotriptan Related Compound D and Almotriptan N-Dimer* test.

<sup>c</sup> The sum of all impurities from the test for *Organic Impurities* and the *Limit of Almotriptan Related Compound D and Almotriptan N-Dimer* test.

#### • LIMIT OF FUMARIC ACID

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.8.

**Mobile phase:** Methanol and Buffer (5:95)

**Standard solution:** 0.0085 mg/mL of USP Fumaric Acid RS and 0.0017 mg/mL of USP Maleic Acid RS in water. Sonication may be used to promote dissolution.

**Sample solution:** 2.8 mg/mL of Almotriptan Malate in water. Sonication may be used to promote dissolution.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Flow rate:** 0.7 mL/min

**Injection volume:** 10 μL

**Run time:** NLT 1.6 times the retention time of fumaric acid

#### System suitability

**Sample:** *Standard solution*

[NOTE—See Table 3 for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between fumaric acid and maleic acid

**Relative standard deviation:** NMT 5.0% for fumaric acid from six injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of fumaric acid (C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) in the portion of Almotriptan Malate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of fumaric acid from the *Sample solution*

$r_S$  = peak response of fumaric acid from the *Standard solution*

$C_S$  = concentration of USP Fumaric Acid RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Almotriptan Malate in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 3.



Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Malic acid <sup>a</sup>	0.60	—
Maleic acid <sup>a</sup>	0.80	—
Fumaric acid	1.0	0.2

<sup>a</sup>Included for identification purposes only.

### SPECIFIC TESTS

- **WATER DETERMINATION**, *Method 1a* (921): NMT 0.5%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in well-closed containers. Store at controlled room temperature.

- **USP REFERENCE STANDARDS** (11)

USP Almotriptan Malate RS

USP Almotriptan Related Compound B RS

2-[(5-[(Pyrrolidin-1-ylsulfonyl)methyl]-1*H*-indol-3-yl)ethanamine hemifumarate.

$C_{15}H_{22}N_3O_2S \cdot \frac{1}{2}C_4H_4O_4$  365.46

USP Almotriptan Related Compound C RS

*N*-Methyl-2-[(5-[(pyrrolidin-1-ylsulfonyl)methyl]-1*H*-indol-3-yl)ethanamine.

$C_{16}H_{23}N_3O_2S$  321.44

USP Almotriptan Related Compound D RS

1-[[[3-[2-(Dimethylamino)ethyl]indol-5-yl)methyl]sulfonyl]pyrrolidine *N*-oxide.

$C_{17}H_{25}N_3O_3S$  351.46

USP Fumaric Acid RS

USP Maleic Acid RS

## Almotriptan Tablets

### DEFINITION

Almotriptan Tablets contain an amount of almotriptan malate ( $C_{17}H_{25}N_3O_2S \cdot C_4H_4O_5$ ) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of almotriptan ( $C_{17}H_{25}N_3O_2S$ ).

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197F)

**Sample**: Sonicate 5 powdered Tablets in 25 mL of water. Extract the suspension with 25 mL of methylene chloride, and discard the organic phase. Add another 25 mL of methylene chloride and 3 mL of 1 N sodium hydroxide. Extract the precipitated base into the organic layer. Dry with anhydrous sodium sulfate, and evaporate the organic solvent. Prepare the residue oil as a film on a sodium chloride pellet.

**Acceptance criteria**: The IR spectrum obtained from the *Sample* is consistent with that of similarly prepared USP Almotriptan Malate RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

- **PROCEDURE**

Protect samples, the Reference Standards, and solutions containing them from light.

**Buffer**: Add 10 mL of triethylamine to every 1000 mL of 0.01 M phosphoric acid. Adjust with phosphoric acid to a pH of 6.0.

**Mobile phase**: Acetonitrile and *Buffer* (10:90)

**Standard solution**: 0.5 mg/mL of USP Almotriptan Malate RS in *Mobile phase*. Sonication may be used to aid in dissolution.

**System suitability stock solution**: 0.1 mg/mL each of USP Almotriptan Related Compound B RS, USP Almotriptan Related Compound C RS, and USP Almotriptan Related Compound D RS in methanol. Sonication may be used to aid in dissolution.

**System suitability solution**: 0.001 mg/mL each of USP Almotriptan Related Compound B RS, USP Almotriptan Related Compound C RS, and USP Almotriptan Related Compound D RS prepared from the *System suitability stock solution* in *Standard solution*

**Sample solution**: Nominally 0.5 mg/mL of almotriptan from Tablets prepared as follows. Transfer NLT 8 Tablets into a suitable volumetric flask, and add 80% of the flask volume of *Mobile phase*. Sonicate for NLT 10 min, and dilute with *Mobile phase* to volume. Stir for 30 min, and centrifuge. Pass a portion of the supernatant through a suitable filter of 0.45- $\mu$ m pore size. Use the filtrate.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode**: LC

**Detector**: UV 210 nm

**Column**: 2.1-mm  $\times$  10-cm; 1.8- $\mu$ m packing L1

**Column temperature**: 40°

**Flow rate**: 0.55 mL/min

**Injection volume**: 3  $\mu$ L

### System suitability

**Samples**: *Standard solution* and *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

### Suitability requirements

**Resolution**: NLT 1.5 between almotriptan related compound C and almotriptan peaks, *System suitability solution*

**Tailing factor**: NMT 3.0, *Standard solution*

**Relative standard deviation**: NMT 2.0%, *Standard solution*

### Analysis

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of almotriptan ( $C_{17}H_{25}N_3O_2S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of almotriptan from the *Sample solution*

$r_S$  = peak response of almotriptan from the *Standard solution*

$C_S$  = concentration of USP Almotriptan Malate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of almotriptan in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of almotriptan, 335.46

$M_{r2}$  = molecular weight of almotriptan malate, 469.55

**Acceptance criteria**: 90.0%–110.0% of the labeled amount of almotriptan

### PERFORMANCE TESTS

- **DISSOLUTION** (711)

**Medium**: 0.1 N hydrochloric acid; 900 mL

**Apparatus 2**: 50 rpm

**Time**: 15 min

**Standard solution**: ( $L/600$ ) mg/mL of USP Almotriptan Malate RS in *Medium*, where  $L$  is the label claim in mg/Tablet

**Sample solution**: Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.



**Instrumental conditions****Mode:** UV**Analytical wavelength**

For Tablets labeled to contain 6.25 mg: 228 nm

For Tablets labeled to contain 12.5 mg: 284 nm

**Blank:** Medium**Analysis****Samples:** Standard solution and Sample solutionCalculate the percentage of the labeled amount of almotriptan ( $C_{17}H_{25}N_3O_2S$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

 $A_U$  = absorbance of the Sample solution $A_S$  = absorbance of the Standard solution $C_S$  = concentration of USP Almotriptan Malate RS in the Standard solution (mg/mL) $L$  = label claim (mg/Tablet) $V$  = volume of Medium, 900 mL $M_{r1}$  = molecular weight of almotriptan, 335.46 $M_{r2}$  = molecular weight of almotriptan malate, 469.55**Tolerances:** NLT 80% (Q) of the labeled amount of almotriptan ( $C_{17}H_{25}N_3O_2S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES**• **ORGANIC IMPURITIES**

Mobile phase, Standard solution, System suitability solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

**Analysis****Samples:** Standard solution, System suitability solution, and Sample solution

Chromatograph the System suitability solution and identify the components on the basis of their relative retention times, as shown in Table 1.

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak response of each degradation product from the Sample solution $r_S$  = peak response of almotriptan from the Standard solution $C_S$  = concentration of USP Almotriptan Malate RS in the Standard solution (mg/mL) $C_U$  = nominal concentration of almotriptan in the Sample solution (mg/mL) $M_{r1}$  = molecular weight of almotriptan, 335.46 $M_{r2}$  = molecular weight of almotriptan malate, 469.55**Acceptance criteria:** See Table 1.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Spiroalmotriptan <sup>a</sup>	0.32	0.4
2-Hydroxyalmotriptan <sup>b</sup>	0.47	0.2
Almotriptan related compound B <sup>c</sup>	0.82	—

<sup>a</sup> 1'-Methyl-5-[(pyrrolidin-1-ylsulfonyl)methyl]spiro[indoline-3,3'-pyrrolidin]-2-ol.<sup>b</sup> 3-[2-(Dimethylamino)ethyl]-5-[(pyrrolidin-1-ylsulfonyl)methyl]-1H-indol-2-ol.<sup>c</sup> This is a process impurity that is included in this table for identification only. This impurity is controlled in the drug substance. This impurity is not to be reported for the drug product and is not to be included in the total degradation products.**Table 1 (Continued)**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Almotriptan related compound C <sup>c</sup>	0.93	—
Almotriptan	1.0	—
Almotriptan related compound D	1.39	0.2
Any individual unspecified degradation product	—	0.2
Total degradation products	—	1.0

<sup>a</sup> 1'-Methyl-5-[(pyrrolidin-1-ylsulfonyl)methyl]spiro[indoline-3,3'-pyrrolidin]-2-ol.<sup>b</sup> 3-[2-(Dimethylamino)ethyl]-5-[(pyrrolidin-1-ylsulfonyl)methyl]-1H-indol-2-ol.<sup>c</sup> This is a process impurity that is included in this table for identification only. This impurity is controlled in the drug substance. This impurity is not to be reported for the drug product and is not to be included in the total degradation products.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Almotriptan Malate RS

USP Almotriptan Related Compound B RS

2-[5-[(Pyrrolidin-1-ylsulfonyl)methyl]-1H-indol-3-yl]ethanamine hemifumarate.

 $C_{15}H_{22}N_3O_2S \cdot \frac{1}{2}C_4H_4O$  365.46

USP Almotriptan Related Compound C RS

N-Methyl-2-[5-[(pyrrolidin-1-ylsulfonyl)methyl]-1H-indol-3-yl]ethanamine.

 $C_{16}H_{23}N_3O_2S$  321.44

USP Almotriptan Related Compound D RS

1-[[[3-[2-(Dimethylamino)ethyl]indol-5-yl)methyl]sulfonyl]pyrrolidine N-oxide.

 $C_{17}H_{25}N_3O_3S$  351.46**Aloe****DEFINITION**

Aloe is the dried latex of the leaves of *Aloe vera* (L.) Burm. f. (syn. *Aloe barbadensis* Mill.), known in commerce as aloe vera, Curaçao aloe, or Barbados aloe; or of *Aloe ferox* Mill., or of hybrids of *Aloe ferox* Mill. with *Aloe africana* Mill. and *Aloe spicata* L.f., known in commerce as cape aloe (Fam. Liliaceae). Aloe vera contains NLT 16.0% of aloin, and cape aloe and its hybrids contain NLT 6.0% of aloin, both calculated on the dried basis.

**IDENTIFICATION**• **A.****Sample:** 1 g finely powdered**Analysis:** Mix the Sample with 25 mL of cold water.

Shake the mixture occasionally during 2 h, filter, and wash the filter and residue with sufficient cold water to make the filtrate measure 100 mL.

**Acceptance criteria:** The color of the filtrate, viewed in the bulb of a 100-mL volumetric flask, is dark orange with curaçao aloe and greenish yellow with cape aloe. The filtrate darkens on standing. [NOTE—Reserve the filtrate for Identification test B.]• **B.****Sample:** 5 mL of the filtrate obtained in Identification test A



**Analysis:** Add 2 mL of nitric acid to the *Sample*, and mix.

**Acceptance criteria:** The mixture exhibits a reddish-orange color with aloe vera and a reddish-brown color that changes rapidly to green with cape aloe.

#### • C. THIN-LAYER CHROMATOGRAPHY

**Standard solution:** 1.0 mg/mL of USP Aloin RS in methanol

**Sample solution:** 0.5 g of finely powdered Aloe in 10 mL of methanol, sonicate for 15 min, centrifuge or filter, and use the supernatant or the filtrate.

#### Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

**Adsorbent:** Chromatographic silica gel mixture with an average particle size of 5  $\mu$ m (HPTLC plates)

**Application volume:** 2  $\mu$ L of the *Standard solution* and 5  $\mu$ L of the *Sample solution* as 8-mm bands

**Relative humidity:** Condition the plate to a relative humidity of about 33% using a suitable device.

**Developing solvent system:** Ethyl acetate, methanol, and water (100:17:13)

**Developing distance:** 6 cm

**Derivatization reagent:** 10% solution of potassium hydroxide in methanol (prepare in an ice bath)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Apply the samples as bands to a suitable high performance thin-layer chromatographic plate. Use a saturated chamber. Develop the chromatograms, dry in air, derivatize with *Derivatization reagent*, and heat at 110° for 5 min. Examine under visible light and UV light at 365 nm.

**Acceptance criteria:** Under visible light, the *Sample solution* chromatogram exhibits a brown band due to aloin at about the middle of the chromatogram, corresponding in color and  $R_f$  to the band exhibited by the *Standard solution*. *Sample solution* containing aloe vera exhibits an additional violet band due to 7-hydroxyaloin right below the aloin band. *Sample solution* containing cape aloe lacks the violet band due to 7-hydroxyaloin. Under UV light at 365 nm, the *Sample solution* chromatogram exhibits a yellow fluorescence band due to aloin, corresponding in color and  $R_f$  to the band exhibited by the *Standard solution*, and a light blue fluorescence band due to aloesine at about one third of the chromatogram.

#### ASSAY

##### • CONTENT OF ALOIN

**Mobile phase:** A mixture of acetonitrile and water (3:7)

**Standard solution:** 0.1 mg/mL of USP Aloin RS in methanol and water (1:1)

**Sample solution:** Transfer about 0.1 g of aloe vera or 0.2 g of cape aloe, finely powdered and accurately weighed, to a 100-mL volumetric flask, and add about 75 mL of methanol. Sonicate for 30 min, cool to room temperature, adjust to volume using methanol, and mix. Before injection, pass through a PTFE membrane filter of 0.45- $\mu$ m pore size, discarding the first few mL of the filtrate.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 295 nm

**Column:** 4.6-mm  $\times$  25-cm; end-capped 5- $\mu$ m, packing L1

**Column temperature:** 43  $\pm$  1°

**Flow rate:** 1.0 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for the aloin peak

**Column efficiency:** NLT 2000 theoretical plates for the aloin peak

**Relative standard deviation:** NMT 2.0% determined from the aloin peak in repeated injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—*Standard solution* and *Sample solution* are stable for 8 h at room temperature.]

Using the chromatogram of *Standard solution*, identify the retention time of the peak corresponding to aloin in the *Sample solution*.

Calculate the percentage of aloin in the portion of Aloe taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

$r_U$  = peak area for aloin from the *Sample solution*

$r_S$  = peak area for aloin from the *Standard solution*

$C_S$  = concentration of USP Aloin RS in the *Standard solution* (mg/mL)

$V$  = final volume of the *Sample solution* (mL)

$W$  = weight of Aloe taken to prepare the *Sample solution* (mg)

**Acceptance criteria:** Aloe vera contains NLT 16.0% of aloin, and cape aloe and its hybrids contain NLT 6.0% of aloin, both calculated on the dried basis.

#### • WATER-SOLUBLE EXTRACTIVE

**Sample:** 2 g of powdered Aloe

**Analysis:** Macerate the *Sample* in 70 mL of water in a suitable flask. Shake the mixture during 8 h at 30-min intervals, and allow it to stand for 16 h without shaking. Filter, and wash the flask and residue with small portions of water, passing the washings through the filter until the filtrate measures 100.0 mL. Evaporate a 50-mL aliquot of the filtrate in a tared dish on a steam bath to dryness, and dry at 110° to constant weight.

**Acceptance criteria:** The weight of water-soluble extractive so obtained is NLT 50% of the weight of Aloe taken.

#### SPECIFIC TESTS

##### • LOSS ON DRYING (731)

**Sample:** Use a powdered sample. If the Aloe is not powdered, crush it in a mortar until it passes through a no. 40 sieve, and mix the ground material before weighing the sample.

**Analysis:** Dry at 105° for 5 h.

**Acceptance criteria:** NMT 12.0%

##### • ARTICLES OF BOTANICAL ORIGIN, Total Ash (561)

**Acceptance criteria:** NMT 4.0%

##### • ALCOHOL-INSOLUBLE SUBSTANCES

**Sample:** 1 g of powdered Aloe

**Analysis:** Add the *Sample* to 50 mL of alcohol in a flask. Heat the mixture to boiling, and maintain at incipient boiling for 15 min, replacing any loss due to evaporation. Remove from the heat, and shake the mixture at intervals during 1 h. Pass through a small dried and tared filter paper or a dried and tared filtering crucible, and wash the residue on the filter with alcohol until the last washing is colorless. Dry the residue at 105° to constant weight.

**Acceptance criteria:** The weight of the residue is NMT 10.0% of the weight of Aloe taken.



### BOTANIC CHARACTERISTICS

**Curaçao aloë:** Brownish black, opaque masses. Its fractured surface is uneven, waxy, and somewhat resinous.

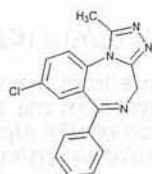
**Cape aloë:** Dusky to dark brown irregular masses, the surfaces of which are often covered with a yellowish powder. Its fracture is smooth and glassy.

**Powdered aloë:** Yellow, yellowish brown to olive-brown in color. When mounted in olive oil, it appears as greenish-yellow to reddish-brown irregular fragments, the hues of which depend to some extent upon the thickness of the fragments.

### ADDITIONAL REQUIREMENTS

- **USP REFERENCE STANDARDS** (11)  
USP Aloin RS

## Alprazolam



$C_{17}H_{13}ClN_4$  308.76  
4H-[1,2,4]Triazolo[4,3- $\alpha$ ][1,4]benzodiazepine, 8-chloro-1-methyl-6-phenyl-;  
8-Chloro-1-methyl-6-phenyl-4H-s-triazolo[4,3- $\alpha$ ][1,4]benzodiazepine [28981-97-7].

### DEFINITION

Alprazolam contains NLT 98.0% and NMT 102.0% of  $C_{17}H_{13}ClN_4$ .

**[CAUTION]**—Care should be taken to prevent inhaling particles of Alprazolam and exposing the skin to it.]

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B.** The retention time of the major peak from the *Sample solution* corresponds to that from the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Diluent:** Acetonitrile and water (1:1)

**Buffer:** 1.4 g/L of monobasic potassium phosphate in water

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Standard solution:** 25  $\mu$ g/mL of USP Alprazolam RS in *Diluent*. [NOTE—The solution is stable for 48 h at room temperature when stored in closed containers.]

**Sample solution:** 25  $\mu$ g/mL of Alprazolam in *Diluent*. Sonicate for about 1 min. [NOTE—The solution is stable for 48 h at room temperature when stored in closed containers.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 231 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of alprazolam ( $C_{17}H_{13}ClN_4$ ) in the portion of Alprazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Alprazolam in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0%

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.5%

#### Delete the following:

- **HEAVY METALS**, *Method II* (231): 20 ppm • (Official 1-Jan-2018)

#### ORGANIC IMPURITIES

**Diluent, Buffer, Mobile phase, and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability solution:** 20  $\mu$ g/mL each of USP Alprazolam RS, USP Alprazolam Related Compound A RS, and USP 2-Amino-5-chlorobenzophenone RS in *Diluent*

**Standard solution:** 0.25  $\mu$ g/mL of USP Alprazolam RS in *Diluent*. [NOTE—When stored in closed containers, the solution is stable for 48 h at room temperature.]

**Sample solution:** 250  $\mu$ g/mL of Alprazolam in *Diluent*.

Sonicate for about 1 min. [NOTE—When stored in closed containers, the *Sample solution* is stable for 24 h at room temperature.]

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—For relative retention times, see *Table 1*.]

#### Suitability requirements

**Resolution:** NLT 2.0 between alprazolam related compound A and alprazolam, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Alprazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each impurity in the *Sample solution*

$r_S$  = peak response for alprazolam from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = concentration of Alprazolam in the *Sample solution* ( $\mu$ g/mL)

$F$  = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*.



Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alprazolam related compound A	0.8	0.76	0.15
Alprazolam	1.0	1.0	—
2-Amino-5-chlorobenzophenone	4.0	1.0	0.15
Individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

**SPECIFIC TESTS**

- **LOSS ON DRYING (731):** Dry a sample at 105° for 1 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Alprazolam RS  
USP Alprazolam Related Compound A RS  
2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3H-1,4-benzodiazepine.  
USP 2-Amino-5-chlorobenzophenone RS  
2-Amino-5-chlorobenzophenone.  
C<sub>13</sub>H<sub>10</sub>ClNO 231.68

## Alprazolam Compounded Oral Suspension

**DEFINITION**

Alprazolam Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>).

Prepare Alprazolam Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations (795)*).

Alprazolam	100 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

Commingle tablets in a suitable mortar to a fine powder, or add *Alprazolam* powder. Add about 20 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

**ASSAY**• **PROCEDURE**

**Buffer:** 0.04 M sodium acetate solution. Adjust with glacial acetic acid to a pH of 2.4.

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (45:8:47)

**Standard solution:** 20 µg/mL of USP Alprazolam RS in *Mobile phase*

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Dilute a suitable vol-

ume of the Oral Suspension in *Mobile phase* to obtain a nominal concentration of 20 µg/mL.

**Chromatographic system**

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 0.6 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time of alprazolam is about 10 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 1.4% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of alprazolam in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- **pH (791):** 4.0–5.0

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature, or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded when stored at controlled room temperature or in a refrigerator
- **LABELING:** Label it to state that it is to be well-shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS (11)**  
USP Alprazolam RS

## Alprazolam Tablets

**DEFINITION**

Alprazolam Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam (C<sub>17</sub>H<sub>13</sub>ClN<sub>4</sub>).

**IDENTIFICATION**• **A. INFRARED ABSORPTION**

**Sample:** An amount of finely powdered Tablets, equivalent to 15 mg of alprazolam, prepared as follows. Dissolve the *Sample* in 10 mL of 10 mg/mL of sodium carbonate solution. Add 15 mL of chloroform, and shake vigorously for 30 min. Centrifuge, withdraw the aqueous layer, and transfer the chloroform to a clean container. Add 200 mg of potassium bromide. Evaporate the chloroform from this mixture to dryness, and dry the dispersion in vacuum at 60° for 24 h. Grind this dispersion into a fine powder. Prepare a suitable pellet for testing by placing 100 mg of dried potassium bromide into a die. Sprinkle 20 mg of the finely ground alprazolam–potassium bromide dispersion onto the dried potassium bromide layer, and cover with another specimen of 100 mg of dried potassium bromide.

**Acceptance criteria:** The IR absorption spectrum of the potassium bromide dispersion so obtained exhibits



maxima characteristic of alprazolam, as compared to that of a similar preparation of USP Alprazolam RS, at the following wavenumbers: at 1609, 1578, 1566, 1539, 1487, and 1379 wavenumbers in the region of 1650–1300  $\text{cm}^{-1}$ ; at 932, 891, 826, 779, 746, 696, and 658 wavenumbers in the region of 975–600  $\text{cm}^{-1}$ .

## ASSAY

### PROCEDURE

**Mobile phase:** Acetonitrile, chloroform, butyl alcohol, glacial acetic acid, and water (850:80:50:0.5:20)

**Internal standard solution:** 0.25 mg/mL of triazolam in acetonitrile

**Standard stock solution:** 0.25 mg/mL of USP Alprazolam RS in *Internal standard solution*

**Standard solution:** 25  $\mu\text{g/mL}$  of USP Alprazolam RS from *Standard stock solution* in acetonitrile

**Sample solution:** Nominally 25  $\mu\text{g/mL}$  of alprazolam from finely powdered Tablets (NLT 20) prepared as follows. Transfer a suitable amount of the powdered tablets to a suitable volumetric flask. Add 1% of the flask volume of water. Transfer 10% of the flask volume of *Internal standard solution*, shake vigorously for 10 min, and dilute with acetonitrile to volume.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  30-cm; packing L3

**Flow rate:** 2 mL/min

**Injection size:** 20  $\mu\text{L}$

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Resolution:** NLT 2.0 between triazolam and alprazolam

**Relative standard deviation:** NMT 2.0% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ( $\text{C}_{17}\text{H}_{13}\text{ClN}_4$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak area response ratio of the alprazolam peak relative to the internal standard peak from the *Sample solution*

$R_S$  = peak area response ratio of the alprazolam peak relative to the internal standard peak from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of alprazolam in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

### DISSOLUTION, Procedure for a Pooled Sample (711)

**Buffer stock solution:** Dissolve 80 g of monobasic potassium phosphate and 20 g of dibasic potassium phosphate in 1 L of water. Add, with mixing, phosphoric acid or potassium hydroxide solution (45 in 100), as necessary to adjust the solution, such that the resulting solution has a pH of  $6.0 \pm 0.1$ .

**Buffer:** Prepare a 1-in-10 dilution of the *Buffer stock solution* to obtain a solution that has a pH of  $6.0 \pm 0.1$ .

**Medium:** *Buffer*; 500 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Buffer* (35:5:60)

**Standard stock solution:** 0.05 mg/mL of USP Alprazolam RS in methanol

**Standard solution:** Add 50 mL of *Buffer stock solution* and 250 mL of water to a 500-mL flask. Add to the flask 5.0 mL of *Standard stock solution* for every 0.25 mg of alprazolam contained in the Tablet being assayed. Dilute with water to volume.

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  10-cm; packing L7

**Flow rate:** 1 mL/min

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Column efficiency:** NLT 500 theoretical plates

**Relative standard deviation:** NMT 3.0% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of labeled amount of alprazolam ( $\text{C}_{17}\text{H}_{13}\text{ClN}_4$ ) dissolved.

**Tolerances:** NLT 80% (Q) of the labeled amount of alprazolam ( $\text{C}_{17}\text{H}_{13}\text{ClN}_4$ ) is dissolved.

### UNIFORMITY OF DOSAGE UNITS (905)

**Mobile phase, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Internal standard solution:** 0.032 mg/mL of triazolam in acetonitrile

**Standard solution:** 0.025 mg/mL of USP Alprazolam RS in *Internal standard solution*

**Sample solution:** Transfer 1 Tablet to a container. Add 0.4 mL of water directly onto the Tablet, allow the Tablet to stand for 2 min, and then swirl the container to disperse the Tablet. For every 0.25 mg of alprazolam contained in the Tablet, add 10.0 mL of *Internal standard solution* to the container. Shake, and centrifuge if necessary.

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of alprazolam ( $\text{C}_{17}\text{H}_{13}\text{ClN}_4$ ) in the Tablet taken:

$$\text{Result} = (R_U/R_S) \times C \times V \times (100/L)$$

$R_U$  = peak area response ratio of the alprazolam peak relative to the internal standard peak from the *Sample solution*

$R_S$  = peak area response ratio of the alprazolam peak relative to the internal standard peak from the *Standard solution*

$C$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

$V$  = volume of *Internal standard solution* used to prepare the *Sample solution* (mL)

$L$  = label claim (mg/Tablet)

**Acceptance criteria:** Meet the requirements

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Alprazolam RS



## Alprazolam Extended-Release Tablets

### DEFINITION

Alprazolam Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### Add the following:

- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.  $\Delta_{USP40}$

### ASSAY

### Change to read:

#### • PROCEDURE

**Mobile phase:** Acetonitrile, water, and phosphoric acid (350:650:1)

**Standard solution:** 0.05 mg/mL of USP Alprazolam RS in methanol

**Sample solution:** Nominally 0.05 mg/mL of alprazolam prepared as follows. Transfer an appropriate number of Tablets to a suitable volumetric flask. Sonicate in 80% of the flask volume of methanol for 15 min, and shake mechanically for 30 min. Dilute with methanol to final volume, filter a portion of the solution, and discard the first 3 mL of filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm.  $\Delta$ For Identification B, use a diode array detector in the range of 200–400 nm.  $\Delta_{USP40}$

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Column efficiency:** NLT 3000 theoretical plates

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of alprazolam in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

### Change to read:

#### • DISSOLUTION (711)

##### Test 1

**Medium:** pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic po-

tassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of  $6.0 \pm 0.1$ ); 500 mL

**Apparatus 1:** 100 rpm

**Times:** 1, 4, 8, and 12 h

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Medium* (7:1:12)

**Standard stock solution:** 0.5 mg/mL of USP Alprazolam RS in acetonitrile

**Standard solution:** (L/500) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu$ m packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 100  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Column efficiency:** NLT 3000 theoretical plates

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 500 mL

**Tolerances:** See *Table 1*.

Table 1

Time (h)	Amount Dissolved		
	0.5-mg Tablet (%)	2-mg Tablet (%)	3-mg Tablet (%)
1	NMT 25	NMT 20	NMT 20
4	40–60	30–55	30–55
8	70–90	65–90	65–90
12	NLT 85	NLT 85	NLT 85

The percentages of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) released at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of  $6.0 \pm 0.1$ ); 500 mL

**Apparatus 1:** 100 rpm

**Times:** 1, 4, 8, and 16 h

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Medium* (35:5:60)

**Standard stock solution:** 0.05 mg/mL of USP Alprazolam RS in methanol

**Standard solution:** (L/500) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 7.5-cm; 5-μm packing L7**Flow rate:** 1.3 mL/min**Injection volume:** 80 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the concentration ( $C_i$ ) of alprazolam ( $C_{17}H_{13}ClN_4$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result}_i = (r_u/r_s) \times C_s$$

 $r_u$  = peak response of alprazolam from the *Sample solution* at each time point $r_s$  = peak response of alprazolam from the *Standard solution* $C_s$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_s)] + (C_i \times V_s)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_s)]] + [(C_2 + C_i) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times [V - (3 \times V_s)]] + [(C_3 + C_2 + C_i) \times V_s]\} \times (1/L) \times 100$$

 $C_i$  = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL) $V$  = volume of *Medium*, 500 mL $L$  = label claim (mg/Tablet) $V_s$  = volume of the *Sample solution* withdrawn at each time point (ERR 1-Jun-2016) (mL)**Tolerances:** See Table 2.**Table 2**

Time Point (i)	Time (h)	Amount Dissolved			
		0.5-mg Tablet (%)	1-mg Tablet (%)	2-mg Tablet (%)	3-mg Tablet (%)
1	1	NMT 25	NMT 25	NMT 20	NMT 20
2	4	45–60	40–55	30–50	25–45
3	8	70–90	65–85	55–75	50–70
4	16	NLT 85	NLT 85	NLT 85	NLT 80

The percentages of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) released at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.**Medium:** pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of 6.0 ± 0.1); 500 mL, deaerated**Apparatus 1:** 100 rpm**Times:** 1, 4, and 8 h for Tablets labeled to contain 0.5 mg or 1 mg; 1, 4, 8, and 16 h for Tablets labeled to contain 2 mg or 3 mg**Mobile phase:** Acetonitrile and *Medium* (40:60)**Standard stock solution:** 0.5 mg/mL of USP Alprazolam RS in methanol**Standard solution:** (L/500) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where L is the label claim in mg/Tablet**Sample solution:** Pass a portion of the solution under test through a suitable filter of 1-μm pore size.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 10-cm; 3-μm or 5-μm packing L7**Flow rate:** 1 mL/min**Injection volume:** 100 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 5.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the concentration ( $C_i$ ) of alprazolam ( $C_{17}H_{13}ClN_4$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result}_i = (r_u/r_s) \times C_s$$

 $r_u$  = peak response of alprazolam from the *Sample solution* at each time point $r_s$  = peak response of alprazolam from the *Standard solution* $C_s$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_s)] + (C_i \times V_s)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_s)]] + [(C_2 + C_i) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times [V - (3 \times V_s)]] + [(C_3 + C_2 + C_i) \times V_s]\} \times (1/L) \times 100$$

 $C_i$  = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL) $V$  = volume of *Medium*, 500 mL $L$  = label claim (mg/Tablet) $V_s$  = volume of the *Sample solution* withdrawn at each time point (ERR 1-Jun-2016) (mL)**Tolerances:** See Table 3.**Table 3**

Time Point (i)	Time (h)	Amount Dissolved			
		0.5-mg Tablet (%)	1-mg Tablet (%)	2-mg Tablet (%)	3-mg Tablet (%)
1	1	15–35	10–30	10–30	5–25
2	4	50–75	45–65	30–55	25–50
3	8	NLT 75	NLT 70	60–80	50–75
4	16	—	—	NLT 85	NLT 80



The percentages of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) released at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Medium:** pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of 6.0); 500 mL

**Apparatus 1** (20-mesh basket): 100 rpm

**Times:** 1, 4, 8, and 16 h

**Mobile phase:** Acetonitrile and *Medium* (32:68)

**Standard stock solution:** 0.4 mg/mL of USP Alprazolam RS in methanol

**Standard solution:** ( $L/500$ ) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where  $L$  is the label claim in mg/Tablet. Pass through a suitable filter of 0.45- $\mu$ m pore size, and use the filtrate.

**Sample solution:** At the end of specified time intervals, withdraw a known volume ( $V_s$ ) of the solution from the dissolution vessel, and replace an equal volume of fresh *Medium* into the dissolution vessel. Pass the withdrawn sample through a suitable filter of 0.45- $\mu$ m pore size, and use the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 100  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration ( $C_i$ ) of alprazolam ( $C_{17}H_{13}ClN_4$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result}_i = (r_u/r_s) \times C_s$$

$r_u$  = peak response of alprazolam from the *Sample solution* at each time point

$r_s$  = peak response of alprazolam from the *Standard solution*

$C_s$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_s)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times V] + [(C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times V] + [(C_3 + C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$C_i$  = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

$V$  = volume of *Medium*, 500 mL

$L$  = label claim (mg/Tablet)

$V_s$  = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

**Tolerances:** See *Table 4*.

**Table 4**

Time Point (i)	Time (h)	Amount Dissolved			
		0.5-mg Tablet (%)	1-mg Tablet (%)	2-mg Tablet (%)	3-mg Tablet (%)
1	1	NMT 40	NMT 35	NMT 35	NMT 35
2	4	50–75	45–65	35–55	30–55
3	8	NLT 75	70–90	55–75	50–70
4	16	NLT 85	NLT 85	NLT 85	NLT 75

The percentages of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) released at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

**Test 5:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

**Medium:** pH 6.0 phosphate buffer (8.0 g/L of monobasic potassium phosphate and 2.0 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 6.0); 500 mL

**Apparatus 1:** 100 rpm

**Times:** 1, 4, 8, and 16 h

**Mobile phase:** Acetonitrile, water, and phosphoric acid (350:650:1)

**Standard stock solution:** 0.5 mg/mL of USP Alprazolam RS in methanol

**Standard solution:** ( $L/500$ ) mg/mL of USP Alprazolam RS in *Medium* from the *Standard stock solution*, where  $L$  is the label claim in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size, and use the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration ( $C_i$ ) of alprazolam ( $C_{17}H_{13}ClN_4$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result}_i = (r_u/r_s) \times C_s$$

$r_u$  = peak response of alprazolam from the *Sample solution* at each time point

$r_s$  = peak response of alprazolam from the *Standard solution*

$C_s$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)



Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) dissolved at each time point (i):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times (V - V_s)) + (C_1 \times V_s)] \times (1/L) \times 100$$

$$\text{Result}_3 = ((C_3 \times [V - (2 \times V_s)]) + [(C_2 + C_1) \times V_s]) \times (1/L) \times 100$$

$$\text{Result}_4 = ((C_4 \times [V - (3 \times V_s)]) + [(C_3 + C_2 + C_1) \times V_s]) \times (1/L) \times 100$$

$C_i$  = concentration of alprazolam in the *Sample solution* at the specified time point (mg/mL)

$V$  = volume of *Medium*, 500 mL

$L$  = label claim (mg/Tablet)

$V_s$  = volume of the *Sample solution* withdrawn at each time point (mL)

Tolerances: See *Table 5*.

**Table 5**

Time Point (i)	Time (h)	Amount Dissolved (%)
1	1	NMT 25
2	4	40–65
3	8	65–95
4	16	NLT 85

The percentages of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) released at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

**Buffer:** 5.4 g/L of monobasic potassium phosphate ( $KH_2PO_4$ ) in water. Adjust with phosphoric acid to a pH of 3.4.

**Solution A:** Acetonitrile, methanol, and *Buffer* (27:10:63)

**Solution B:** Acetonitrile, methanol, and *Buffer* (7:3:10)

**Mobile phase:** See *Table 6*.

**Table 6**

Time (min)	Solution A (%)	Solution B (%)
0	95	5
22	95	5
25	15	85
60	15	85
60.1	95	5
70	95	5

**System suitability solution:** 1 µg/mL each of USP Chlordiazepoxide Related Compound A RS, USP Alprazolam Related Compound A RS, and USP Nordazepam RS; and 0.4 µg/mL of USP Alprazolam RS in methanol

**Standard solution:** 0.4 µg/mL of USP Alprazolam RS in methanol

**Sample solution:** From NLT 20 Tablets ground to a fine powder, transfer an amount of powder to a suitable flask to obtain a nominal concentration of 0.2 mg/mL of alprazolam in methanol. [NOTE—Sonicate for 15 min to dissolve the contents.] Filter a portion, and discard the first 1 mL of filtrate.

## Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 µL

## System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are listed in *Table 7*.]

## Suitability requirements

**Resolution:** NLT 1.5 between nordazepam and alprazolam; NLT 1.5 between chlordiazepoxide related compound A and alprazolam related compound A, *System suitability solution*

**Tailing factor:** NMT 2.0 for the alprazolam peak, *System suitability solution*

**Relative standard deviation:** NMT 5%, *Standard solution*

## Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of the impurity from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of alprazolam in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 7*)

**Acceptance criteria:** See *Table 7*.

**Table 7**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlordiazepoxide related compound A <sup>a</sup>	0.36	1.0	0.2
Alprazolam related compound A	0.45	0.7	0.5
Nordazepam <sup>a,b</sup>	0.8	1.0	0.2
Alprazolam	1.0	—	—
2-Amino-5-chloro-benzophenone	1.8	0.9	0.5
Amino-derivative <sup>c</sup>	2.2	1.2	0.5
Any other individual degradation product	—	1.0	0.2
Total impurities	—	—	2.0

<sup>a</sup> If possible from the manufacturing process.

<sup>b</sup> 7-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one.

<sup>c</sup> 7-Chloro-1-methyl-5-phenyl[1,2,4]triazolo[4,3-a]quinolin-4-amine.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.

- **LABELING:** The labeling states the *Dissolution* test used only if *Test 1* is not used.



**Change to read:**• **USP REFERENCE STANDARDS (11)**

- USP Alprazolam RS
- USP Alprazolam Related Compound A RS
- 2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3H-1,4-benzodiazepine.
- $C_{17}H_{13}ClN_4O$  326.78
- USP Chlordiazepoxide Related Compound A RS
- 7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.
- $C_{15}H_{11}ClN_2O_2$  286.71  $\Delta_{USP40}$
- USP Nordazepam RS

## Alprazolam Orally Disintegrating Tablets

**DEFINITION**

Alprazolam Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ).

**IDENTIFICATION****Change to read:**

- $\Delta_{USP40}$  The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**Add the following:**

- $\Delta_{USP40}$  **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.  $\Delta_{USP40}$

**ASSAY****Change to read:**• **PROCEDURE**

- Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.
- Diluent:** Acetonitrile and water (60:40)
- Mobile phase:** Acetonitrile, methanol, and *Buffer* (35:10:55)
- Standard solution:** 10  $\mu$ g/mL of USP Alprazolam RS in *Diluent*
- Sample solution:** Nominally 10  $\mu$ g/mL of alprazolam from Tablets prepared as follows. Transfer 10 Tablets to a suitable volumetric flask. Add *Diluent* to volume and pass through a suitable filter. [NOTE—Sonicate with intermittent shaking to help dissolve, if necessary.]
- Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)
- Mode:** LC
- Detector:** UV 221 nm.  $\Delta$ For *Identification B*, use a diode array detector in the range of 200–400 nm.  $\Delta_{USP40}$

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection volume:** 30  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of alprazolam in the *Sample solution* ( $\mu$ g/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**• **DISINTEGRATION (701)**

**Test 1**

**Time:** NMT 60 s

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Disintegration Test 2*.

**Time:** NMT 30 s

**Change to read:**• **DISSOLUTION (711)**

**Test 1**

**Medium:** pH 6.0 phosphate buffer (8 g/L of monobasic potassium phosphate and 2 g/L of dibasic potassium phosphate in water. Adjust with phosphoric acid or diluted potassium hydroxide to a pH of  $6.0 \pm 0.1$ ); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 10 min

**Mobile phase, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*, except use an *Injection volume* of 100  $\mu$ L.

**Standard stock solution:**  $\Delta$ 0.05 mg/mL  $\Delta_{USP40}$  of USP Alprazolam RS in methanol. [NOTE—Sonicate to help dissolve, if necessary.]

**Standard solution:**  $\Delta$ (L/1000) mg/mL  $\Delta_{USP40}$  of USP Alprazolam RS  $\Delta$ from the *Standard stock solution* in *Medium*,  $\Delta_{USP40}$  where L is the label claim in  $\Delta$ mg/ *Tablet*,  $\Delta_{USP40}$

**Sample solution:** Pass a portion of the solution under test through a nylon membrane filter of 0.45- $\mu$ m pore size, discarding the first few mL.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/ *Tablet*)

**Tolerances:** NLT 80% (Q) of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** pH 6.0 phosphate buffer (8 g/L of monobasic potassium phosphate and 2 g/L of dibasic potas-



sium phosphate in water. Adjust with phosphoric acid or potassium hydroxide to a pH of  $6.0 \pm 0.1$ ; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 10 min

**Buffer:** 1.36 g/L of monobasic potassium phosphate.

Adjust with dilute sodium hydroxide to a pH of 6.0.

**Mobile phase:** Acetonitrile and Buffer (35:65)

**Standard stock solution:**  $\Delta 0.05$  mg/mL<sub>USP40</sub> of USP Alprazolam RS in methanol. [NOTE—Sonicate to help dissolve, if necessary.]

**Standard solution:**  $\Delta(L/500)$  mg/mL<sub>USP40</sub> of USP Alprazolam RS  $\Delta$ from the *Standard stock solution* in *Medium*<sub>USP40</sub> where *L* is the label claim in  $\Delta$ mg/*Tablet*<sub>USP40</sub>

**Sample solution:** Pass a 5-mL aliquot of the solution under test through a suitable filter of 0.45- $\mu$ m pore size, discarding the first 3 mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm  $\times$  7.5-cm; 5- $\mu$ m packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 40  $\mu$ L

**Run time:** 3 times the retention time of alprazolam

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 500 mL

$L$  = label claim (mg/*Tablet*)

**Tolerances:** NLT 70% (Q) of the labeled amount of alprazolam ( $C_{17}H_{13}ClN_4$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Diluent:** Prepare as directed in the *Assay*.

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

**Solution A:** Acetonitrile, methanol, and Buffer (25:20:55)

**Solution B:** Acetonitrile, methanol, and Buffer (40:5:55)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
12	100	0
15	0	100
60	0	100
65	100	0
70	100	0

**Standard solution:** 0.6  $\mu$ g/mL of USP Alprazolam RS in *Diluent*

**Sample solution:** Nominally 200  $\mu$ g/mL of alprazolam in *Diluent*. Prepare using 10 *Tablets*, and pass through a suitable filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7

**Column temperature:** 30°

**Flow rate:** 1.2 mL/min

**Injection volume:** 25  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Theoretical plates:** NLT 2000

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 6.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of *Tablets* taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of alprazolam from the *Standard solution*

$C_S$  = concentration of USP Alprazolam RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of alprazolam in the *Sample solution* ( $\mu$ g/mL)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*. Disregard any peaks less than 0.05%.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Alprazolam related compound A <sup>a,b</sup>	0.8	—	—
Alprazolam	1.0	—	—
2-Amino-5-chlorobenzophenone	2.9	1.9	0.5
Any other unknown impurity	—	1.0	0.5
Total impurities	—	—	2.0

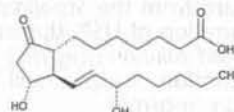
<sup>a</sup> 2-(2-Acetylhydrazino)-7-chloro-5-phenyl-3H-1,4-benzodiazepine.

<sup>b</sup> Disregard the peak due to alprazolam related compound A, because it is a process impurity in alprazolam.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** When more than one *Disintegration* test is given, the labeling states the *Disintegration* test used only if *Test 1* is not used. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**  
USP Alprazolam RS

## Alprostadil



$C_{20}H_{34}O_5$

354.48



Prost-13-en-1-oic acid, 11,15-dihydroxy-9-oxo-, (11 $\alpha$ ,13E,15S)-;  
(1R,2R,3R)-3-Hydroxy-2-[(E)-(3S)-3-hydroxy-1-octenyl]-5-oxocyclopentaneheptanoic acid [745-65-3].

## DEFINITION

Alprostadil contains NLT 95.0% and NMT 105.0% of alprostadil (C<sub>20</sub>H<sub>34</sub>O<sub>5</sub>), calculated on the anhydrous basis.

**[CAUTION]**—Great care should be taken to prevent inhaling particles of Alprostadil and exposing the skin to it.]

## IDENTIFICATION

### • A. INFRARED ABSORPTION (197M)

## ASSAY

### • PROCEDURE

Use freshly prepared solutions.

**Mobile phase:** Methanol, acetonitrile, and 0.1 M monobasic potassium phosphate (2:1:2). Adjust with phosphoric acid to a pH of 3.0.

**Diluent:** Methanol and water (90:10)

**Internal standard solution:** 0.05 mg/mL of ethylparaben in *Diluent*

**Standard stock solution:** 0.3 mg/mL of USP Alprostadil RS in *Diluent*

**Standard solution:** 0.2 mg/mL of USP Alprostadil RS prepared by combining 2.0 mL of *Standard stock solution* with 1.0 mL of *Internal standard solution*

**System suitability stock solution:** 4.5  $\mu$ g/mL of USP Prostaglandin A<sub>1</sub> RS in *Standard solution*

**System suitability solution:** Combine 2.0 mL of *System suitability stock solution* with 1.0 mL of *Internal standard solution*.

**Sample stock solution:** 0.3 mg/mL of Alprostadil in *Diluent*

**Sample solution:** 0.2 mg/mL of Alprostadil prepared by combining 2.0 mL of *Sample stock solution* and 1.0 mL of *Internal standard solution*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Photodiode array detector or equivalent capable of detecting UV wavelengths of 200–300 nm

**Analytical wavelength:** 200 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

### System suitability

**Sample:** *System suitability solution*

### Suitability requirements

**Resolution:** NLT 7.5 between prostaglandin A<sub>1</sub> and alprostadil, and NLT 2.0 between prostaglandin A<sub>1</sub> and ethylparaben

**Relative standard deviation:** NMT 2.0%, determined from the peak area ratio of alprostadil to ethylparaben

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of alprostadil (C<sub>20</sub>H<sub>34</sub>O<sub>5</sub>) in the portion of Alprostadil taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak area ratio of alprostadil to the internal standard from the *Sample solution*

$R_S$  = peak area ratio of alprostadil to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Alprostadil RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Alprostadil in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0% on the anhydrous basis

## IMPURITIES

### • RESIDUE ON IGNITION (281)

**Sample:** 0.3 g

**Acceptance criteria:** NMT 0.5%

### • LIMIT OF CHROMIUM

**Standard stock solution:** 3.04  $\mu$ g/mL of chromium trichloride in 0.05 M nitric acid

**Standard solution:** 20 ng/mL of chromium (Cr) in alcohol, prepared as follows. Transfer 2 mL of the *Standard stock solution* to a 100-mL volumetric flask, and dilute with alcohol to volume.

**Sample solution:** 1.0 mg/mL of Alprostadil in alcohol

**Blank:** Alcohol

### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectroscopy

**Lamp:** Chromium hollow-cathode

**Analytical wavelength:** 357.9 nm

**Atomization type:** Graphite furnace

### Temperatures

**Drying:** 100°

**Ashing:** 1000°

**Atomization:** 2700°

**Injection volume:** 20  $\mu$ L

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of chromium (Cr) in the portion of Alprostadil taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of chromium in the *Standard solution* (mg/mL)

$C_U$  = concentration of Alprostadil in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.002%

### • LIMIT OF RHODIUM

**Standard stock solution:** 100  $\mu$ g/mL of rhodium in 1.2 M hydrochloric acid, prepared by diluting rhodium chloride hydrate

**Standard solution:** 50 ng/mL of rhodium (Rh) in alcohol from *Standard stock solution*

**Sample solution:** 2.0 mg/mL of Alprostadil in alcohol

**Blank:** Alcohol

### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectroscopy

**Lamp:** Rhodium hollow-cathode

**Analytical wavelength:** 343.5 nm

**Atomization type:** Graphite furnace

### Temperatures

**Drying:** 100°

**Ashing:** 1000°

**Atomization:** 2800°

**Injection volume:** 20  $\mu$ L

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of rhodium (Rh) in the portion of Alprostadil taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of rhodium in the *Standard solution* (mg/mL)

$C_U$  = concentration of Alprostadil in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.002%

### • LIMIT OF FOREIGN PROSTAGLANDINS, TEST 1

Use freshly prepared solutions.



**Mobile phase:** Methanol, acetonitrile, and 0.1 M monobasic potassium phosphate (2:1:2). Adjust with phosphoric acid to a pH of 3.0.

**Diluent:** Methanol and water (90:10)

**Standard solution:** 6 µg/mL of USP Alprostadil RS, 15 µg/mL of USP Prostaglandin A<sub>1</sub> RS, and 6 µg/mL of USP Prostaglandin B<sub>1</sub> RS in *Diluent*

**Sample solution:** 3.0 mg/mL of Alprostadil in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Photodiode array detector or equivalent capable of detecting UV wavelengths of 200–300 nm

**Analytical wavelengths**

Prostaglandin A<sub>1</sub>: 224 nm

Prostaglandin B<sub>1</sub>: 280 nm

Other foreign prostaglandins: 200 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 7.5 between prostaglandin A<sub>1</sub> and alprostadil

**Relative standard deviation:** NMT 4.0%, determined from the peaks at their respective wavelength for replicate injections

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of prostaglandin A<sub>1</sub> and prostaglandin B<sub>1</sub> in the portion of Alprostadil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of prostaglandin A<sub>1</sub> or prostaglandin B<sub>1</sub> from the *Sample solution*

$r_S$  = peak response of prostaglandin A<sub>1</sub> or prostaglandin B<sub>1</sub> from the *Standard solution*

$C_S$  = concentration of USP Prostaglandin A<sub>1</sub> RS or USP Prostaglandin B<sub>1</sub> RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Alprostadil in the *Sample solution* (mg/mL)

Calculate the percentage of each impurity occurring at 200 nm and eluting before prostaglandin A<sub>1</sub> in the portion of Alprostadil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response for alprostadil from the *Standard solution*

$C_S$  = concentration of USP Alprostadil RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Alprostadil in the *Sample solution* (mg/mL)

Calculate the percentage of the impurity having a relative retention time of 0.6, relative to the prostaglandin A<sub>1</sub> peak detected at 224 nm, in the portion of Alprostadil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for any impurity having a relative retention time of 0.6, relative to the prostaglandin A<sub>1</sub> peak, from the *Sample solution*

$r_S$  = peak response for prostaglandin A<sub>1</sub> from the *Standard solution*

$C_S$  = concentration of USP Prostaglandin A<sub>1</sub> RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Alprostadil in the *Sample solution* (mg/mL)

#### Acceptance criteria

Prostaglandin A<sub>1</sub>: NMT 1.5%

Prostaglandin B<sub>1</sub>: NMT 0.1%

Any foreign prostaglandin impurity eluting before prostaglandin A<sub>1</sub>: NMT 0.9%

Impurity at relative retention time 0.6, relative to prostaglandin A<sub>1</sub>: NMT 0.9%

#### • LIMIT OF FOREIGN PROSTAGLANDINS, TEST 2

**Mobile phase:** Methanol, acetonitrile, and 0.02 M monobasic potassium phosphate (2:1:1). Adjust with phosphoric acid to a pH of 3.

**System suitability solution:** 6 µg/mL of USP Alprostadil RS, 15 µg/mL of USP Prostaglandin A<sub>1</sub> RS, and 6 µg/mL of USP Prostaglandin B<sub>1</sub> RS in methanol and water (9:1)

**Standard solution:** 10 µg/mL of USP Alprostadil RS in acetonitrile and water (1:1)

**Sample solution:** 5.0 mg/mL of Alprostadil in acetonitrile and water (1:1). [NOTE—Sonicate if necessary.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Photodiode array detector or equivalent, capable of detecting UV wavelengths of 200–300 nm

**Analytical wavelengths**

Prostaglandin A<sub>1</sub>: 224 nm

Prostaglandin B<sub>1</sub>: 280 nm

Other foreign prostaglandins: 200 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for alprostadil and prostaglandin A<sub>1</sub> are 1.0 and 1.2, respectively, *System suitability solution*.]

**Suitability requirements**

**Resolution:** NLT 4.0 between prostaglandin A<sub>1</sub> and alprostadil, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, determined from the main peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity, excluding prostaglandin B<sub>1</sub>, observed at 200 nm and eluting after prostaglandin A<sub>1</sub> in the portion of Alprostadil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response for alprostadil from the *Standard solution*

$C_S$  = concentration of USP Alprostadil RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Alprostadil in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Sum of the impurities at relative retention times 2.0 and 2.3:** NMT 0.6%

**Any other foreign prostaglandin impurity eluting after prostaglandin A<sub>1</sub>:** NMT 0.9%

**Total impurities:** The sum of the impurities from *Limit of Foreign Prostaglandins, Test 1* and *Test 2*, is NMT 2.0%.

#### SPECIFIC TESTS

##### • WATER DETERMINATION, Method I (921)

**Sample:** 0.5 g

**Acceptance criteria:** NMT 0.5%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store in a refrigerator.



• **USP REFERENCE STANDARDS (11)**

USP Alprostadil RS  
USP Prostaglandin A<sub>1</sub> RS  
USP Prostaglandin B<sub>1</sub> RS  
(13E,15S)-15-Hydroxy-9-oxoprostano-8(12),13-dien-1-oic acid.  
C<sub>20</sub>H<sub>32</sub>O<sub>4</sub> 336.47

## Alprostadil Injection

### DEFINITION

Alprostadil Injection is a sterile solution of Alprostadil in Dehydrated Alcohol. It contains NLT 90.0% and NMT 115.0% of the labeled amount of alprostadil (C<sub>20</sub>H<sub>34</sub>O<sub>5</sub>).

### IDENTIFICATION

• **A. INFRARED ABSORPTION (197K)**

**Sample:** Dry an amount of Injection, equivalent to 2 mg of alprostadil, on 500 mg of spectroscopic grade potassium bromide at 40°–50° under vacuum. Prepare a pellet from this mixture.

**Standard:** A solution of USP Alprostadil RS dissolved in dehydrated alcohol and processed as described in the *Sample*

**Acceptance criteria:** Meets the requirements

### ASSAY

• **PROCEDURE**

**Solution A:** 40 mg/mL of α-bromo-2'-acetonaphthone in acetonitrile, freshly prepared

**Solution B:** 5 mg/mL of diisopropylethylamine in acetonitrile, freshly prepared

**Mobile phase:** Methylene chloride, 1,3-butanediol, and water (1000:6:0.5)

**Internal standard solution:** 50 µg/mL of ethylparaben in methylene chloride

**Standard stock solution:** 0.5 mg/mL of USP Alprostadil RS in dehydrated alcohol

**Standard solution:** 0.13 mg/mL of USP Alprostadil RS, prepared as follows. Gently evaporate a 0.5-mL portion of the *Standard stock solution* to dryness with a stream of nitrogen. Add 150 µL of *Solution A*, rinse the inside of the container with this solution, and swirl. Add 150 µL of *Solution B* to the container, rinse the inside of the container with this solution, and swirl. Cap and sonicate to dissolve. Heat the container at 45° for 45 min, swirling occasionally. Sonicate again after heating is complete. Discard the specimen if the entire sample does not dissolve. Evaporate the solution using a stream of nitrogen, add 2.0 mL of *Internal standard solution*, and sonicate to dissolve. Discard the specimen if the entire sample does not dissolve.

**Sample solution:** Nominally 0.13 mg/mL of alprostadil, prepared as follows. Pool the contents of several containers of the Injection. Gently evaporate a volume, equivalent to 0.25 mg of alprostadil, to dryness using a stream of nitrogen. Proceed as directed for the *Standard solution*, beginning with "Add 150 µL of *Solution A*".

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.4-mm × 25-cm; packing L18

**Flow rate:** 1.5 mL/min

**Injection volume:** Equal volumes of *Standard solution* and *Sample solution*

### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for ethylparaben and alprostadil are about 0.4 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 9.0 between alprostadil and the internal standard

**Relative standard deviation:** NMT 2.5%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alprostadil (C<sub>20</sub>H<sub>34</sub>O<sub>5</sub>) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of alprostadil to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of alprostadil to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Alprostadil RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of alprostadil the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–115.0%

### SPECIFIC TESTS

• **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 5 USP Endotoxin Units/100 µg of alprostadil.

• **STERILITY TESTS (71):** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **WATER DETERMINATION, Method I (921):** NMT 0.4%

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, single-dose containers, preferably of Type I glass. Store in a refrigerator.

• **USP REFERENCE STANDARDS (11)**

USP Alprostadil RS  
USP Endotoxin RS

## Alteplase

```

      SYQVICRDEK TQMIYQHQHS WLRPLVRSNR VEYCWNSGR AQCHSVPVKS
      CSEPRCFNGG TCQALYFSD FVCQPEGA GKCCIEDTRA TCYEDQGISY
      RGTWTAESG AECTNNSSA LAQPKYSRR PDAIRLGLGN HNYCRNPORD
      SKPWCVFKA GKYSSEFCST PACSEGNSDC YFGNGSAVRG THSLTESGAS
      CLPWNSMILI GKVYTAQNPQ AQALGLGKHN YCRNPQDAK PWCVLKNRR
      LTWEYCDVPS CSTGLRQYS QPQFR

      IKGGLFADIA SHPWQAIFA KHRSPGERF LCGGILISSC WLSAAHCFQ
      ERFPFPHLTV ILGRYRVVP GEEQKFEVE KYIVHKEFDD DTYNDIALI
      QLKSDSSRCA QESSVVRTVC LPPADLQLPD WTECELSGYG KHEALSPFYS
      ERLKEAHVRL YPSSRCTSQH LLNRTVTDNM LCAGDTRSGG PQANLHDAQ
      GDSGGLVLCL NDGRMTLVGI ISWGLGCGQK DVPGVYTKVT NYLDWIRDNM
      RP
  
```

C<sub>2569</sub>H<sub>3894</sub>N<sub>746</sub>O<sub>781</sub>S<sub>40</sub>  
[105857-23-6].

59,007.61

### DEFINITION

Alteplase is a highly purified glycosylated serine protease with fibrin-binding properties and plasminogen-specific proteolytic activities. It is produced by recombinant DNA synthesis in mammalian cell culture. It has a biological potency of NLT 90.0% and NMT 115.0% of the potency stated on the label, the potency being 580,000 USP Alteplase Units/mg of protein.



The presence of host cell DNA and host cell protein impurities in Alteplase is process specific; the limits of these impurities are determined by validated methods.

## IDENTIFICATION

### A.

**Standard solution:** 1.0–2.5 mg/mL of USP Alteplase RS

**Sample solution:** Prepare similarly to the *Standard solution*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

To each of three test tubes transfer 1 mL of 0.5-mg/mL H-D-isoleucyl-prolyl-arginyl-*p*-nitroaniline dihydrochloride. Separately transfer 200  $\mu$ L of the *Standard solution* and 200  $\mu$ L of the *Sample solution* to two of the test tubes. To the third test tube add 200  $\mu$ L of 0.2 M arginine solution that has been adjusted with phosphoric acid to a pH of 7.3 (negative control). Mix the solutions in the three test tubes, and allow to stand for 1 min.

**Acceptance criteria:** A yellow color is produced in the solutions from the *Standard solution* and the *Sample solution*, while no yellow color is produced in the negative control.

### B. PEPTIDE MAPPING

**Solution A:** 6.9 mg/mL of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.85. Filter, and degas.

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
91	70	30
121	40	60
131	40	60

**Dialysis solution:** 480 mg/mL of urea, 44 mg/mL of tris(hydroxymethyl)aminomethane, and 0.88 mg/mL of edetic acid in water. Adjust with hydrochloric acid to a pH of 8.6.

**Standard solution:** Prepare a solution containing 1.0 mg/mL of USP Alteplase RS in water. Dialyze 2.0 mL of this solution into the *Dialysis solution* at room temperature for NLT 12 h. Measure the volume of the solution, and transfer it to a clean test tube. For each mL of solution in the tube, add 10  $\mu$ L of 1 M dithiothreitol. Incubate at room temperature for 4 h, then add 25  $\mu$ L of 1 M iodoacetic acid per mL of the solution, and incubate in the dark for 30 min. Quench the reaction by adding 50  $\mu$ L of 1 M dithiothreitol per mL of the solution. Dialyze the solution against 0.1 M ammonium bicarbonate for 24 h, replacing the 0.1 M ammonium bicarbonate twice during the dialysis period. To 2.0 mL of the dialyzed solution, add 20  $\mu$ g of trypsin, and incubate for 6–8 h at room temperature. Again add 20  $\mu$ g of trypsin, and incubate for 16–18 h for a total of 24 h of incubation of the trypsin-treated solution.

[NOTE—Store the *Standard solution* in a freezer.]

**Sample solution:** Using an accurately weighed quantity of Alteplase, proceed as directed in the *Standard solution*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm  $\times$  10-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 100  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between peaks 6 and 7 as defined by the USP Alteplase RS Data Sheet. The times for peaks 6 and 7 baseline widths are NMT 0.5 min.

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and a mixture of the *Standard solution* and the *Sample solution* (1:1)

Measure the responses for NLT 20 major peaks as defined in the USP Alteplase RS Data Sheet.

**Acceptance criteria:** The retention times of the corresponding peaks of the *Standard solution* and the *Sample solution* do not differ by more than 0.4 min, and the peak area ratios relative to peak 19 (as shown on the USP Alteplase RS Data Sheet) do not differ by more than 20%. No additional significant peaks or shoulders are found, a significant peak or shoulder being defined as one having a peak response of NLT 5% of peak 19.

## ASSAY

### BIOLOGICAL POTENCY

**Buffer:** 1.38 mg/mL of monobasic sodium phosphate, 7.10 mg/mL of anhydrous dibasic sodium phosphate, 0.20 mg/mL of sodium azide, and 0.10 mg/mL of polysorbate 80 in water

**Human thrombin solution:** 33 U.S. Units in terms of the U.S. Standard Thrombin/mL in *Buffer*

**Human fibrinogen solution:** 2 mg/mL of human fibrinogen in *Buffer*

**Human plasminogen solution:** 1 mg/mL of human plasminogen in *Buffer*

**Standard stock solution:** 1.0 mg/mL (580,000 USP Alteplase Units) of USP Alteplase RS in water

**Standard solutions:** Dilute volumes of *Standard stock solution* with water to obtain a series of five *Standard solutions* having known concentrations ranging from 145 to 9.3 USP Alteplase Units/mL.

**Sample stock solution:** 1.0 mg/mL of Alteplase in water

**Sample solutions:** Dilute a volume of *Sample stock solution* with *Buffer* to obtain a series of dilutions of about 1:20,000; 1:10,000; and 1:5,000.

#### Analysis

**Samples:** *Standard solutions* and *Sample solutions*

To a set of labeled glass test tubes add 0.5 mL of *Human thrombin solution*. To separate test tubes add 0.5 mL of each *Standard solution* or *Sample solution*, and store on ice. To a second set of labeled glass tubes, add 20  $\mu$ L of *Human plasminogen solution* and 1 mL of *Human fibrinogen solution*, and store on ice. Beginning with the thrombin–*Standard solution* mixture containing the *Standard solution* with the lowest number of USP Units/mL, record the time, and separately add 200  $\mu$ L of each of the thrombin–*Standard solution* mixtures to the test tubes containing the plasminogen–fibrinogen mixture. Using a vortex mixer, intermittently mix the contents of each tube for a total of 15 s, and carefully place into a rack in a 37° circulating water bath. A visually turbid clot forms within 30 s, followed by the formation of bubbles within the clot. Record the clot lysis time ( $t_d$ ) from the first addition of the Alteplase solution to the last bubble to rise to the surface.

Using a least squares fit, determine the equation of the line using the log values of the standard concentra-



tion, in USP Alteplase Units/mL, versus the log values of their clot lysis times in s taken:

$$\log t = m(\log U_s) + b$$

- $t$  = time to bubble release (s)  
 $m$  = slope of the line  
 $U_s$  = activity of the *Standard solution* (USP Alteplase Units/mL)  
 $b$  = y-intercept of the line

The correlation coefficient is NLT  $-0.9900$ . From the line equation and using the log of the clot lysis time for the *Sample solution*, calculate the log of the activity ( $U_A$ ):

$$\log U_A = \{[(\log t) - b]/m\}$$

Calculate the alteplase activity in USP Alteplase Units/mL taken:

$$\text{Result} = D(10^{\log U})$$

- $D$  = dilution factor for the *Sample solution*  
 Calculate the specific activity in the portion of Alteplase taken:

$$\text{Result} = (U_A/P)$$

- $P$  = concentration of protein obtained in the test for *Protein Content*

**Acceptance criteria:** 90.0%–115.0% of the potency stated on the label, the potency being 580,000 USP Alteplase Units/mg of protein

## OTHER COMPONENTS

### • PROTEIN CONTENT

**Arginine solution:** 34.8 mg/mL of arginine in water. Adjust with phosphoric acid to a pH of 7.3.

**Sample stock solution:** 1 mg/mL of Alteplase in water

**Sample solution:** Dilute a volume of *Sample stock solution* with a volume of *Arginine solution* to obtain a solution having an absorbance value of 0.5–1.0 at the wavelength of maximum absorbance at about 280 nm. Determine the dilution volume ( $V$ ).

### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV

**Wavelength range:** 240–500 nm

**Analytical wavelengths:** 320 nm and maximum absorbance at about 280 nm

**Cell:** 1 cm

**Blank:** *Arginine solution*

### Analysis

**Samples:** *Sample solution* and *Blank*

Calculate the protein content in the portion of Alteplase taken:

$$\text{Result} = [(A_{\max} - A_{320})/\epsilon] \times V$$

- $A_{\max}$  = absorbance value at the wavelength of maximum absorbance  
 $A_{320}$  = absorbance of the *Sample solution* at 320 nm  
 $\epsilon$  = molar absorptivity of Alteplase, 1.9  
 $V$  = volume of *Arginine solution* required to prepare the *Sample solution*

## IMPURITIES

### • CHROMATOGRAPHIC PURITY

**SDS buffer:** 400 mg/mL of glycerol, 5.52 mg/mL of tris(hydroxymethyl)aminomethane hydrochloride, 3.28 mg/mL of tris(hydroxymethyl)aminomethane, 0.20 mg/mL of bromophenol blue, and 0.20 mg/mL of xylene cyanole FF in sodium dodecyl sulfate solution (8 in 100)

**Diluted SDS buffer:** Dilute 1 volume of *SDS buffer* with 4 volumes of water.

**Running buffer:** 3.03 mg/mL of tris(hydroxymethyl)aminomethane and 14.26 mg/mL of glycine in sodium dodecyl sulfate (1 in 1000)

**Carboxymethylation buffer:** 480 mg/mL of urea, 44 mg/mL of tris(hydroxymethyl)aminomethane, and 1.2 mg/mL of edetic acid in water. Adjust with hydrochloric acid, if necessary, to a pH of 8.6.

**Ammoniacal silver nitrate solution:** Transfer 105 mL of sodium hydroxide solution (0.36 in 100) and 7.0 mL of ammonium hydroxide to a 500-mL volumetric flask, and add slowly, with stirring, 20.0 mL of silver nitrate solution (20 in 100). Dilute with water to volume.

[NOTE—Prepare this solution immediately before use, and protect it from light. This amount of solution is sufficient for two slab gels.]

**Citric acid-formaldehyde solution:** To 500 mL of water add 25 mg of citric acid, 0.25 mL of formaldehyde, and 0.025 mL of methanol, omitting the methanol if the formaldehyde is preserved with methanol. [NOTE—Prepare this solution fresh at the time of use.

This amount of solution is sufficient for two slab gels.]

**Gel:** Prepare a 10% T (total acrylamide)–0.25% C (cross-linked bisacrylamide) resolving gel containing 0.1% sodium dodecyl sulfate, 0.375 M tris(hydroxymethyl)aminomethane hydrochloride, and 0.05 M tris(hydroxymethyl)aminomethane.

**Arginine solution:** 34.8 mg/mL of arginine in water. Adjust with phosphoric acid to a pH of 7.3.

**Molecular weight standard solution:** Use a commercially available preparation of low molecular weight protein standards (10,000–100,000 Da) at 2 mg/mL. Mix 990  $\mu$ L of *Diluted SDS buffer* and 10  $\mu$ L of the molecular weight standard mixture.

**Control solution:** Prepare a control solution of bovine serum albumin containing 10  $\mu$ g/mL. For a 10 ng/25  $\mu$ L load, mix 600  $\mu$ L of *Diluted SDS buffer* and 25  $\mu$ L of the control solution, and heat at 90° for 2 min. For a 2.5 ng/25  $\mu$ L load, mix 594  $\mu$ L of *Diluted SDS buffer* and 6  $\mu$ L of the control solution, and heat at 90° for 2 min.

**Standard stock solution:** 1 mg/mL of USP Alteplase RS in water

**Standard solution:** Dilute an accurately measured volume of *Standard stock solution* in *Arginine solution* to obtain a solution having a final concentration of 0.25 mg/mL. Heat 0.5 mL of this solution with 116  $\mu$ L of *SDS buffer* and 10  $\mu$ L of 1 M dithiothreitol at 80° for 2 min.

**Carboxymethylated standard solution:** Dilute 1.0 mL of *Standard stock solution* with 1 mL of *Carboxymethylation buffer*, and adjust with 1 M sodium hydroxide to a pH of 8.5. Add 20  $\mu$ L of 1 M dithiothreitol, and incubate at 37° for 60 min. Add 100  $\mu$ L of 1 M iodoacetic acid, and incubate in the dark for 20 min. Desalt by passing the solution through a chromatographic column containing fine gel chromatographic packing equilibrated with a buffer solution containing 20 mg/mL of sodium dodecyl sulfate, 100 mg/mL of glycerol, 1.42 mg/mL of tris(hydroxymethyl)aminomethane hydrochloride, and 0.85 mg/mL of tris(hydroxymethyl)aminomethane. Collect the protein fraction of the preparation by elution with the same buffer, and add 20  $\mu$ L of 1 M dithiothreitol. Adjust the protein concentration to about 0.2 mg/mL with a buffer solution containing 20 mg/mL of sodium dodecyl sulfate, 100 mg/mL of glycerol, 1.42 mg/mL of tris(hydroxymethyl)aminomethane hydrochloride, 0.85 mg/mL of tris(hydroxymethyl)aminomethane, 1.06 mg/mL of dithiothreitol, 0.05 mg/mL of bromophenol blue, and 0.05 mg/mL of xylene cyanole FF.

**Sample stock solution, Sample solution, and Carboxymethylated sample solution:** Using an accurately weighed quantity of Alteplase, proceed as directed for *Standard stock solution*, *Standard solution*, and *Carboxymethylated standard solution*.



**Blank:** Mix 500  $\mu$ L of water, 126  $\mu$ L of SDS buffer, and 10  $\mu$ L of 1 M dithiothreitol.

#### Analysis

**Samples:** Molecular weight standard solution, Control solution, Standard solution, Carboxymethylated standard solution, Sample solution, Carboxymethylated sample solution, and Blank

Separately apply equal volumes (about 25  $\mu$ L) of the Standard solution, Carboxymethylated standard solution, Sample solution, and Carboxymethylated sample solution at the 5- $\mu$ g load; apply equal volumes (about 38  $\mu$ L) of the Standard solution and the Carboxymethylated standard solution at the 7.5- $\mu$ g load; and apply the Control solutions at the 10- and 2.5-ng load onto separate lanes of the gel. Apply about 25  $\mu$ L of the Molecular weight standard solution to each side of the gel, and apply about 25  $\mu$ L of the Blank onto a separate lane. Apply the Standard solution and the Sample solution on one half and the Carboxymethylated standard solution and Carboxymethylated sample solution on the other half. Perform the electrophoresis using a constant current of 1.3–1.5 mA/cm of gel length and the Running buffer. Remove the gel from the apparatus 10–20 min after the tracking dye starts to move. Place the gel in 250 mL of a solution of 20% alcohol and 6% glacial acetic acid for NLT 1 h, and change the solution every 20 min, leaving the gel to soak overnight following the last change.

Perform silver staining of the gel by placing the gel in 250 mL of a 10% glutaraldehyde solution (v/v) in a shallow dish, and shake for about 30 min. Replace the glutaraldehyde solution with distilled water, allow the gel to soak for about 20 min, and then change the water. Repeat for a total of three washings. Transfer the gel to a dish, and cover with 250 mL of Ammoniacal silver nitrate solution. Place the dish on a shaker for about 15 min. Rinse four times with 250 mL of water, rocking the dish for 1 min between rinses. Continue rocking to prevent the gel from sticking and to facilitate washing.

Transfer the gel to a clear dish containing 250 mL of Citric acid–formaldehyde solution, and rock the dish. Protein bands become visible. When the gel is visibly stained, wash immediately with water, and rinse it repeatedly with water to remove the Citric acid–formaldehyde solution. Rinse the gel for NLT 1 h, and dry. Soak cellophane membranes in glycerol solution (2 in 100). Roll a membrane onto a rigid sheet of plastic. Roll the gel onto the membrane, and cover with another membrane. Lay a frame on the edges of the membranes, and clamp it to the rigid plastic sheet. Dismantle the dryer, and cut off excess cellophane when dry (about 24 h). Visually examine the gel under light.

**System suitability:** The 2.5- and 10-ng Control solutions must be visible. The nonreduced Control solutions migrate with an apparent molecular weight of slightly less than 66,000 Da, as compared with the Molecular weight standard solution.

**Acceptance criteria:** The Sample solution exhibits three major bands in the region between 66,000 Da and 31,000 Da, corresponding to the major bands from the Standard solution. The Carboxymethylated sample solution exhibits six major bands in the region between 92,500 Da and 45,000 Da, corresponding to the major bands from the Carboxymethylated standard solution.

#### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 1 USP Endotoxin Unit/mg of Alteplase
- **SINGLE-CHAIN CONTENT**

**Mobile phase:** 27.6 g of monobasic sodium phosphate in 1000 mL of sodium dodecyl sulfate solution (1 in 1000). Adjust with sodium hydroxide to a pH of 6.8. Filter, and degas.

**Dithiothreitol solution:** 3.12 mg/mL of dithiothreitol in Mobile phase

**Standard stock solution:** Using an accurately weighed quantity of USP Alteplase RS, make a 1-mg/mL solution in water.

**Standard solution:** Pipet 1 mL of the Standard stock solution into a glass tube, add 3 mL of Dithiothreitol solution, cap the tube, and invert to mix. Heat for 3–5 min at about 80°.

**Sample stock solution:** Using an accurately weighed quantity of Alteplase, make a 1-mg/mL solution in water.

**Sample solution:** Pipet 1 mL of the Sample stock solution into a glass tube, add 3 mL of Dithiothreitol solution, cap the tube, and invert to mix. Heat for 3–5 min at about 80°.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 7.5-mm  $\times$  60-cm; packing L25

**Flow rate:** 0.5 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Sample:** Standard solution

#### Suitability requirements

**Resolution:** NLT 1.1 between the single-chain and two-chain alteplase peaks

#### Analysis

**Samples:** Standard solution and Sample solution

[NOTE—The major peaks are from single-chain and two-chain alteplase and from higher and lower molecular weight species.]

Calculate the percentage of single-chain alteplase in the portion of Alteplase taken:

$$\text{Result} = (r_u/r_T) \times 100$$

$r_u$  = peak response for single-chain alteplase

$r_T$  = sum of all the peak responses of alteplase

**Acceptance criteria:** No peaks or shoulders in the Sample solution that are not present in the Standard solution are found; NLT 60%.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store in the frozen state at a temperature of –20° or below.
- **USP REFERENCE STANDARDS (11)**  
USP Alteplase RS  
USP Endotoxin RS

## Alteplase for Injection

#### DEFINITION

Alteplase for Injection is a sterile lyophilized preparation of Alteplase. Its biological activity is NLT 90% and NMT 115% of that stated on the label in USP Alteplase Units. It contains NLT 95% and NMT 111% of the total protein content stated on the label.

#### IDENTIFICATION

- **A.**  
**Standard solution:** 1.0–2.5 mg/mL of USP Alteplase RS in water  
**Sample solution:** Prepare similarly to the Standard solution.  
**Analysis**  
**Samples:** Standard solution and Sample solution  
To each of three test tubes transfer 1 mL of 0.5-mg/mL H-D-isoleucyl-prolyl-arginyl-p-nitroaniline dihydrochloride. Separately transfer 200  $\mu$ L of the Standard solution



and 200  $\mu$ L of the *Sample solution* to two of the test tubes. To the third test tube add 200  $\mu$ L of 0.2 M arginine solution that has been adjusted with phosphoric acid to a pH of 7.3 (negative control). Mix the solutions in the three test tubes, and allow to stand for 1 min.

**Acceptance criteria:** A yellow color is produced in the solutions from the *Standard solution* and the *Sample solution*, while no yellow color is produced in the negative control.

#### • B. PEPTIDE MAPPING

**Solution A:** 6.9 mg/mL of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.85. Filter, and degas.

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
91	70	30
121	40	60
131	40	60

**Dialysis solution:** 480 mg/mL of urea, 44 mg/mL of tris(hydroxymethyl)aminomethane, and 0.88 mg/mL of edetic acid in water. Adjust with hydrochloric acid to a pH of 8.6.

**Standard solution:** Prepare a solution containing 1.0 mg/mL of USP Alteplase RS in water. Dialyze 2.0 mL of this solution into the *Dialysis solution* at room temperature for NLT 12 h. Measure the volume of the solution, and transfer it to a clean test tube. For each mL of solution in the tube, add 10  $\mu$ L of 1 M dithiothreitol. Incubate at room temperature for 4 h, then add 25  $\mu$ L of 1 M iodoacetic acid per mL of the solution, and incubate in the dark for 30 min. Quench the reaction by adding 50  $\mu$ L of 1 M dithiothreitol per mL of the solution. Dialyze the solution against 0.1 M ammonium bicarbonate for 24 h, replacing the 0.1 M ammonium bicarbonate twice during the dialysis period. To 2.0 mL of the dialyzed solution, add 20  $\mu$ g of trypsin, and incubate for 6–8 h at room temperature. Again add 20  $\mu$ g of trypsin, and incubate for 16–18 h for a total of 24 h of incubation of the trypsin-treated solution. [NOTE—Store the *Standard solution* in a freezer.]

**Sample solution:** Using a quantity of Alteplase for Injection, proceed as directed in the *Standard solution*.

#### Chromatographic system

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm  $\times$  10-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 100  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between peaks 6 and 7 as defined by the USP Alteplase RS Data Sheet. The times for peaks 6 and 7 baseline widths are NMT 0.5 min.

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and a mixture of the *Standard solution* and the *Sample solution* (1:1)

Measure the responses for NLT 20 major peaks as defined in the USP Alteplase RS Data Sheet.

**Acceptance criteria:** The retention times of corresponding peaks of the *Standard solution* and the *Sample solution* do not differ by more than 0.4 min, and the peak area ratios relative to peak 19 (as shown on the USP

Alteplase RS Data Sheet) do not differ by more than 20%. No additional significant peaks or shoulders are found, a significant peak or shoulder being defined as one having a peak response of NLT 5% of peak 19.

#### ASSAY

##### • BIOLOGICAL POTENCY

**Buffer:** 1.38 mg/mL of monobasic sodium phosphate, 7.10 mg/mL of anhydrous dibasic sodium phosphate, 0.20 mg/mL of sodium azide, and 0.10 mg/mL of polysorbate 80 in water

**Human thrombin solution:** 33 U.S. Units in terms of the U.S. Standard Thrombin/mL in *Buffer*

**Human fibrinogen solution:** 2 mg/mL of human fibrinogen in *Buffer*

**Human plasminogen solution:** 1 mg/mL of human plasminogen in *Buffer*

**Standard stock solution:** 1.0 mg/mL (580,000 USP Alteplase Units) of USP Alteplase RS in water

**Standard solutions:** Dilute volumes of *Standard stock solution* with water to obtain a series of five *Standard solutions* having known concentrations ranging from 145 to 9.3 USP Alteplase Units/mL.

**Sample stock solution:** 1.0 mg/mL of Alteplase for Injection in water

**Sample solutions:** Dilute a volume of *Sample stock solution* with *Buffer* to obtain a series of dilutions of about 1:20,000; 1:10,000; and 1:5,000.

#### Analysis

**Samples:** *Standard solutions* and *Sample solutions*

To a set of labeled glass test tubes add 0.5 mL of *Human thrombin solution*. To separate test tubes add 0.5 mL of each *Standard solution* or *Sample solution*, and store on ice. To a second set of labeled glass tubes, add 20  $\mu$ L of *Human plasminogen solution* and 1 mL of *Human fibrinogen solution*, and store on ice. Beginning with the thrombin–*Standard solution* mixture containing the *Standard solution* with the lowest number of USP Units/mL, record the time, and separately add 200  $\mu$ L of each of the thrombin–*Standard solution* mixtures to the test tubes containing the plasminogen–fibrinogen mixture. Using a vortex mixer, intermittently mix the contents of each tube for a total of 15 s, and carefully place into a rack in a 37° circulating water bath. A visually turbid clot forms within 30 s, followed by the formation of bubbles within the clot. Record the clot lysis time ( $t_c$ ) from the first addition of the alteplase solution to the last bubble to rise to the surface.

Using a least squares fit, determine the equation of the line using the log values of the standard concentration, in USP Alteplase Units/mL, versus the log values of their clot lysis times in seconds taken:

$$\log t = m(\log U_s) + b$$

$t$  = time to bubble release (s)

$m$  = slope of the line

$U_s$  = activity of the *Standard solution* (USP Alteplase Units/mL)

$b$  = y-intercept of the line

The correlation coefficient is NLT –0.9900. From the line equation and using the log of the clot lysis time for the *Sample solution*, calculate the log of the activity ( $U_A$ ):

$$\log U_A = [(\log t) - b]/m$$

Calculate the alteplase activity in USP Alteplase Units/mL taken:

$$\text{Result} = D(10^{\log U})$$

$D$  = dilution factor for the *Sample solution*



Calculate the specific activity in the portion of Alteplase for Injection taken:

$$\text{Result} = (U_A/P)$$

$P$  = concentration of protein obtained in the test for Protein Content

**Acceptance criteria:** 90%–115% of the potency stated on the label in USP Alteplase Units

## OTHER COMPONENTS

### • PROTEIN CONTENT

**Arginine solution:** 34.8 mg/mL of arginine in water. Adjust with phosphoric acid to a pH of 7.3.

**Sample stock solution:** 1 mg/mL of Alteplase for Injection in water

**Sample solution:** Dilute a volume of *Sample stock solution* with a volume of *Arginine solution* to obtain a solution having an absorbance value of 0.5–1.0 at the wavelength of maximum absorbance at about 280 nm. Determine the dilution volume ( $V$ ).

### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV

**Wavelength range:** 240–500 nm

**Analytical wavelengths:** 320 nm and maximum absorbance at about 280 nm

**Cell:** 1 cm

**Blank:** *Arginine solution*

### Analysis

**Samples:** *Sample solution* and *Blank*

Calculate the protein content in the portion of Alteplase for Injection taken:

$$\text{Result} = [(A_{\text{max}} - A_{320})/\epsilon] \times V$$

$A_{\text{max}}$  = absorbance value at the wavelength of maximum absorbance

$A_{320}$  = absorbance of the *Sample solution* at 320 nm

$\epsilon$  = molar absorptivity of alteplase, 1.9

$V$  = volume of *Arginine solution* required to prepare the *Sample solution*

**Acceptance criteria:** 95%–111% of the total protein content stated on the label

## PERFORMANCE TESTS

### • UNIFORMITY OF DOSAGE UNITS (905)

**Acceptance criteria:** Meets the requirements for *Content Uniformity*

## SPECIFIC TESTS

### • PERCENT MONOMER

**Mobile phase:** 34.84 mg/mL of arginine, 158.56 mg/mL of ammonium sulfate, and 100 mL/L of isopropyl alcohol in water. Adjust with phosphoric acid to a pH of 7.3, degas, and pass through a filter of 0.45- $\mu$ m pore size.

**System suitability solution:** 1 mg/mL each of chicken ovalbumin and bovine gamma globulin

**Standard solution:** 1 mg/mL of USP Alteplase RS in water

**Sample solution:** 1 mg/mL of Alteplase for Injection in water

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 7.5-mm  $\times$  30-cm; packing L25

**Flow rate:** 0.5–1.0 mL/min

**Injection volume:** 50  $\mu$ L

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 1.6 between gamma globulin and ovalbumin, *System suitability solution*

**Column efficiency:** NLT 1200 theoretical plates, determined from the alteplase peak, *Standard solution*

### Analysis

**Sample:** *Sample solution*

Calculate, as a percentage, the monomer in the portion of Alteplase for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of the alteplase monomer

$r_T$  = sum of all the peak responses related to alteplase

**Acceptance criteria:** NLT 95.0%

### • SINGLE-CHAIN CONTENT

**Mobile phase:** 27.6 mg/mL of monobasic sodium phosphate in sodium dodecyl sulfate solution (1 in 1000). Adjust with sodium hydroxide to a pH of 6.8. Filter, and degas.

**Dithiothreitol solution:** 3.12 mg/mL of dithiothreitol in *Mobile phase*

**Standard stock solution:** Using an accurately weighed quantity of USP Alteplase RS, make a 1-mg/mL solution in water.

**Standard solution:** Pipet 1 mL of the *Standard stock solution* into a glass tube. Add 3 mL of *Dithiothreitol solution*, cap the tube, and invert to mix. Heat for 3–5 min at about 80°.

**Sample stock solution:** Using an accurately weighed quantity of Alteplase for Injection, make a 1-mg/mL solution in water.

**Sample solution:** Pipet 1 mL of the *Sample stock solution* into a glass tube. Add 3 mL of *Dithiothreitol solution*, cap the tube, and invert to mix. Heat for 3–5 min at about 80°.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 7.5-mm  $\times$  60-cm; packing L25

**Flow rate:** 0.5 mL/min

**Injection volume:** 50  $\mu$ L

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Resolution:** NLT 1.1 between the single-chain and two-chain alteplase peaks

### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The major peaks are from single-chain and two-chain alteplase and from higher and lower molecular weight species.]

Calculate the percentage of single-chain alteplase in the portion of Alteplase for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for single-chain alteplase

$r_T$  = sum of all the peak responses of alteplase

**Acceptance criteria:** No peaks or shoulders in the *Sample solution* that are not present in the *Standard solution* are found; NLT 60%.

• **INJECTIONS AND IMPLANTED DRUG PRODUCTS (1):** Meets the requirements of constituted solutions at the time of use

• **pH (791)**

**Sample solution:** Constitute as directed in the labeling.

**Acceptance criteria:** 7.1–7.5

• **WATER DETERMINATION (921), Method I:** NMT 4.0%

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 1 USP Endotoxin Unit/mg

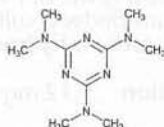
• **STERILITY TESTS (71):** Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*

• **BIOLOGICAL REACTIVITY TESTS, IN VIVO (88):** Meets the requirements for *Safety Tests—Biologicals*



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in hermetic, light-resistant containers, and store in a refrigerator.
- **LABELING:** Label it to state the biological activity in USP Alteplase Units/vial and the amount of protein/vial.
- **USP REFERENCE STANDARDS** (11)  
USP Alteplase RS  
USP Endotoxin RS

**Altretamine**

$C_9H_{18}N_6$  210.28  
1,3,5-Triazine-2,4,6-triamine, *N,N,N',N'',N''*-hexamethyl-;  
Hexamethylmelamine [645-05-6].

**DEFINITION**

Altretamine contains NLT 98.0% and NMT 102.0% of altretamine ( $C_9H_{18}N_6$ ), calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 0.79 g/L of ammonium carbonate in water. Adjust with a solution of formic acid (1 in 10) or ammonium hydroxide (1 in 10) to a pH of  $8.0 \pm 0.05$ .

**Diluent:** Methanol and water (65:35)

**Mobile phase:** Methanol and *Buffer* (65:35)

**Standard stock solution:** 0.5 mg/mL of USP Altretamine RS in a mixture of methanol and water (70:30), prepared by first dissolving the Standard in methanol and then diluting with water to final volume.

**Standard solution:** 0.05 mg/mL of USP Altretamine RS in *Diluent*, from *Standard stock solution*

**Sample stock solution:** Transfer 25 mg of Altretamine to a 50-mL volumetric flask. Dissolve in 35 mL of methanol, and dilute with water to volume.

**Sample solution:** 0.05 mg/mL of Altretamine in *Diluent*, from *Sample stock solution*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 227 nm

**Column:** 4.6-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of altretamine ( $C_9H_{18}N_6$ ) in the portion of Altretamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Altretamine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**Delete the following:**

- **HEAVY METALS, Method II** (231): NMT 40 ppm (Official 1-Jan-2018)

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method I** (921): NMT 1%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Altretamine RS

**Altretamine Capsules****DEFINITION**

Altretamine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of altretamine ( $C_9H_{18}N_6$ ).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)

**Sample:** Remove as completely as possible the contents of 5 Capsules, and dissolve, with shaking, in 10 mL of chloroform. Filter, and evaporate the chloroform solution to dryness.

**Acceptance criteria:** Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 0.79 g/L of ammonium carbonate in water. Adjust with a solution of formic acid (1 in 10) or ammonium hydroxide (1 in 10) to a pH of  $8.0 \pm 0.05$ .

**Diluent:** Methanol and water (65:35)

**Mobile phase:** Methanol and *Buffer* (65:35)

**Standard stock solution:** 0.5 mg/mL of USP Altretamine RS in a mixture of methanol and water (70:30), prepared by first dissolving the Standard in methanol and then diluting with water to final volume.

**Standard solution:** 0.05 mg/mL of USP Altretamine RS in *Diluent*, from *Standard stock solution*

**Sample stock solution:** Remove as completely as possible the contents of NLT 20 Capsules, and weigh. Mix the combined contents, and transfer as completely as possible to a 500-mL volumetric flask. Add 325 mL of methanol, and sonicate. Dilute with water to volume.

**Sample solution:** Transfer a volume of the *Sample stock solution*, equivalent to 10 mg of altretamine, to a 200-mL volumetric flask, and dilute with *Diluent* to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 227 nm

Column: 4.6-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of altretamine ( $C_9H_{18}N_6$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Altretamine RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Detector: UV 242 nm

Standard solution: USP Altretamine RS in Medium

Analysis: Determine the amount of  $C_9H_{18}N_6$  dissolved from UV absorbances on filtered portions of the solution under test, suitably diluted if necessary with Medium, compared with the Standard solution.

Tolerances: NLT 80% (Q) of the labeled amount of  $C_9H_{18}N_6$  is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in tight, light-resistant containers, and store at controlled room temperature.

### • USP REFERENCE STANDARDS (11)

USP Altretamine RS

## Ammonium Alum

$AlNH_4(SO_4)_2 \cdot 12H_2O$  453.33

$AlNH_4(SO_4)_2$  237.15

Sulfuric acid, aluminum ammonium salt (2:1:1), dodecahydrate;

Aluminum ammonium sulfate (1:1:2), dodecahydrate [7784-26-1].

Anhydrous [7784-25-0].

## DEFINITION

Ammonium Alum contains NLT 99.0% and NMT 100.5% of ammonium alum [ $AlNH_4(SO_4)_2$ ], calculated on the dried basis.

## IDENTIFICATION

### • A.

Sample solution: 50 mg/mL

Analysis: Add 1 N sodium hydroxide dropwise to the Sample solution.

Acceptance criteria: A precipitate is formed, and it dissolves in an excess of the reagent with the evolution of ammonia, recognizable by its alkaline effect upon moistened red litmus paper exposed to the vapor.

### • B. IDENTIFICATION TESTS—GENERAL, Aluminum (191)

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

### • C. IDENTIFICATION TESTS—GENERAL, Sulfate (191)

Sample solution: 50 mg/mL

Analysis: Proceed as directed in Identification Tests—General, Sulfate (191), except centrifuge the neutral solutions of sulfates and use the supernatants for the lead acetate test.

Acceptance criteria: Meets the requirements

## ASSAY

### • PROCEDURE

Edetate disodium titrant: Prepare and standardize as directed in Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M).

Sample: 800 mg of Ammonium Alum

Analysis: Transfer the Sample to a 400-mL beaker, moisten with 1 mL of glacial acetic acid, and add 50 mL of water, 50.0 mL of Edetate disodium titrant and 20 mL of acetic acid–ammonium acetate buffer TS. Warm on a steam bath until the solution is complete, and boil gently for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and titrate the excess edetate disodium with 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, and make any necessary correction. Each mL of 0.05 M Edetate disodium titrant is equivalent to 11.86 mg of  $AlNH_4(SO_4)_2$ .

Acceptance criteria: 99.0%–100.5% on the dried basis

## IMPURITIES

Delete the following:

### • HEAVY METALS, Method I (231)

Test preparation: Dissolve 1 g in 20 mL of water, and add 5 mL of 0.1 N hydrochloric acid. Evaporate the solution in a porcelain evaporating dish to dryness. Treat the residue with 20 mL of water, and add 50 mg of hydroxylamine hydrochloride. Heat the solution on a steam bath for 10 min, cool, and dilute with water to 25 mL.

Analysis: Proceed as directed in the chapter, except add 50 mg of hydroxylamine hydrochloride to the Standard Preparation.

Acceptance criteria: 20 ppm (Official 1-Jan-2018)

### • IRON

Sample solution: 6.7 mg/mL

Analysis: Add 5 drops of potassium ferrocyanide TS to 20 mL of the Sample solution.

Acceptance criteria: No blue color is produced immediately.

## SPECIFIC TESTS

### • LOSS ON DRYING (731)

Sample: 2.0 g

Analysis: Transfer the Sample, in a tared porcelain crucible, to a muffle furnace at 200°. Increase the temperature to 300°, and dry at 300° to a constant weight. Cool in a desiccator, and weigh.

Acceptance criteria: 45.0%–48.0%

### • LIMIT OF ALKALIES AND ALKALINE EARTHS

Sample: 1 g

Analysis: Completely precipitate the aluminum from a boiling solution of the Sample in 100 mL of water by the addition of sufficient 6 N ammonium hydroxide to render the solution distinctly alkaline to methyl red TS, and filter. Evaporate the filtrate to dryness, and ignite.

Acceptance criteria: The weight of the residue is NMT 5 mg (0.5%).



## Potassium Alum

$\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	474.39
$\text{AlK}(\text{SO}_4)_2$	258.21
Sulfuric acid, aluminum potassium salt (2:1:1), dodecahydrate;	
Aluminum potassium sulfate (1:1:2) dodecahydrate [7784-24-9].	
Anhydrous [10043-67-1].	

### DEFINITION

Potassium Alum contains NLT 99.0% and NMT 100.5% of potassium alum  $[\text{AlK}(\text{SO}_4)_2]$ , calculated on the dried basis.

### IDENTIFICATION

- **A.**  
**Sample solution:** 50 mg/mL in water  
**Analysis:** Add 1 N sodium hydroxide dropwise to the *Sample solution*.  
**Acceptance criteria:** A precipitate is formed that dissolves in an excess of the reagent. Ammonia is not evolved (distinction from ammonium alum).
- **B.**  
**Analysis:** Hold it in a nonluminous flame.  
**Acceptance criteria:** A violet color is imparted to the flame.
- **C.**  
**Sample solution:** Saturated solution in water  
**Analysis:** Add 10 mL of sodium bitartrate TS to 5 mL of the *Sample solution*.  
**Acceptance criteria:** A white, crystalline precipitate is formed within 30 min.
- **D. IDENTIFICATION TESTS—GENERAL, Aluminum (191) AND Sulfate (191)**  
**Sample solution:** 50 mg/mL in water  
**Acceptance criteria:** Meets the requirements

### ASSAY

- **PROCEDURE**  
**Edetate disodium titrant:** Prepare and standardize as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.  
**Sample:** 800 mg  
**Analysis:** Transfer the *Sample* to a 400-mL beaker, moisten with 1 mL of glacial acetic acid, and add 50 mL of water, 50.0 mL of *Edetate disodium titrant*, and 20 mL of acetic acid–ammonium acetate buffer TS. Warm on a steam bath until solution is complete, and boil gently for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and titrate 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 12.91 mg of potassium alum  $[\text{AlK}(\text{SO}_4)_2]$ .  
**Acceptance criteria:** 99.0%–100.5% on the dried basis

### IMPURITIES

#### Delete the following:

- **HEAVY METALS, Method I (231)**  
**Sample solution:** Dissolve 1 g in water to make 20 mL, and add 5 mL of 0.1 N hydrochloric acid. Evaporate the solution in a porcelain evaporating dish to dryness. Treat the residue with 20 mL of water, and add 50 mg of hydroxylamine hydrochloride. Heat the solution on a steam bath for 10 min, cool, and dilute with water to 25 mL.  
**Analysis:** Proceed as directed, except add 50 mg of hydroxylamine hydrochloride to the *Standard Preparation*.

**Acceptance criteria:** 20 ppm (Official 1-Jan-2018)

#### IRON

**Sample solution:** Potassium alum in water (1 in 150)  
**Analysis:** Add 5 drops of potassium ferrocyanide TS to 20 mL of the *Sample solution*.

**Acceptance criteria:** No blue color is produced immediately.

### SPECIFIC TESTS

#### LOSS ON DRYING (731)

**Sample:** 2.0 g

**Analysis:** Transfer the *Sample* in a tared porcelain crucible to a muffle furnace at 200°. Increase the temperature to 400°, and dry at 400° to constant weight. Cool in a desiccator, and weigh.

**Acceptance criteria:** 43.0%–46.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.

## Alumina and Magnesia Oral Suspension

### DEFINITION

Alumina and Magnesia Oral Suspension is a mixture containing aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and Magnesium Hydroxide  $[\text{Mg}(\text{OH})_2]$ . It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ . It may contain a flavoring agent, and may contain suitable antimicrobial agents.

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Magnesium (191)**  
**Sample solution:** To a solution of 5 g in 10 mL of 3 N hydrochloric acid add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 min, and filter.  
**Acceptance criteria:** The filtrate meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Aluminum (191)**  
**Sample solution:** Wash the precipitate obtained in *Identification test A* with a hot solution containing 20 mg/mL of ammonium chloride, and dissolve the precipitate in hydrochloric acid.  
**Acceptance criteria:** The solution meets the requirements.

### ASSAY

#### ALUMINUM HYDROXIDE

**Edetate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.

**Sample solution:** Transfer a volume of Oral Suspension, previously well shaken in its original container, equivalent to 1200 mg of aluminum hydroxide, to a suitable beaker. Add 20 mL of water, stir, and slowly add 10 mL of hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.

**Analysis:** Pipet 10 mL of the *Sample solution* into a beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat near the boiling point for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solu-*



tion, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 3.900 mg of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

**Acceptance criteria:** 90.0%–110.0%

#### • MAGNESIUM HYDROXIDE

**Sample solution:** Prepare as directed in the Assay for *Aluminum Hydroxide*.

**Analysis:** Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker. Add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol), and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ .

**Acceptance criteria:** 90.0%–110.0%

#### IMPURITIES

##### • CHLORIDE AND SULFATE, Chloride (221)

**Sample solution:** Dissolve 5.0 g in the minimum volume of nitric acid required to achieve complete solution, add 1 mL of acid in excess, then add water to make 100 mL, and filter.

**Acceptance criteria:** NMT 0.14%; a 10-mL portion of the *Sample solution* shows no more chloride than corresponds to 1.0 mL of 0.020 N hydrochloric acid.

##### • CHLORIDE AND SULFATE, Sulfate (221)

**Sample solution:** Dissolve 5.0 g in 5 mL of 3 N hydrochloric acid, with gentle heating. Cool, add water to make 250 mL, and filter.

**Acceptance criteria:** NMT 0.1%; a 20-mL portion of the *Sample solution* shows no more sulfate than corresponds to 0.40 mL of 0.020 N sulfuric acid.

##### • ARSENIC, Method I (211)

**Standard preparation:** Prepare as directed in *Arsenic* (211), except prepare it to contain 5 µg of arsenic instead of 3 µg.

**Test preparation:** Dissolve a portion of Oral Suspension, equivalent to 0.5 g of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , in 20 mL of 7 N sulfuric acid.

**Acceptance criteria:** NMT 10 ppm, based on the aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  content

#### Delete the following:

##### • HEAVY METALS (231)

**Test preparation:** Dissolve a portion of Oral Suspension, equivalent to 0.24 g of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , in 10 mL of 3 N hydrochloric acid with the aid of heat, filter, if necessary, and dilute with water to 25 mL.

**Acceptance criteria:** NMT 83 ppm, based on the aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  content. (Official 1-Jan-2018)

#### SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** Its total aerobic microbial count does not exceed  $10^2$  cfu/mL, and it meets the requirements for the absence of *Escherichia coli*.

• **pH (791):** 7.3–8.5

##### • ACID-NEUTRALIZING CAPACITY (301)

**Acceptance criteria:** The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M)$$

$F_A$  = theoretical acid-neutralizing capacity of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , 0.0385 mEq

$A$  = amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  in the specimen tested, based on the labeled quantity (mg)

$F_M$  = theoretical acid-neutralizing capacity of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , 0.0343 mEq

$M$  = amount of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$  in the specimen tested, based on the labeled quantity (mg)

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.

• **LABELING:** Oral Suspension may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

## Alumina and Magnesia Tablets

#### DEFINITION

Alumina and Magnesia Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ .

#### IDENTIFICATION

##### • A. IDENTIFICATION TESTS—GENERAL, Magnesium (191)

**Sample solution:** To a 0.7-g portion of finely powdered Tablets add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution changes to deep yellow. Continue boiling for 2 min, and filter.

**Acceptance criteria:** The filtrate meets the requirements.

##### • B. IDENTIFICATION TESTS—GENERAL, Aluminum (191)

**Sample solution:** Wash the precipitate obtained in Identification test A with hot ammonium chloride solution (1 in 50), and dissolve the precipitate in hydrochloric acid.

**Acceptance criteria:** The solution meets the requirements.

#### ASSAY

##### • ALUMINUM HYDROXIDE

**Edetate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.

**Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 1200 mg of aluminum hydroxide, to a 150-mL beaker. Add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.

**Analysis:** Pipet 10 mL of the *Sample solution* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat near the boiling point for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* is equivalent to 3.900 mg of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .



Acceptance criteria: 90.0%–110.0%

• **MAGNESIUM HYDROXIDE**

**Sample solution:** Prepare as directed in the Assay for Aluminum Hydroxide.

**Analysis:** Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker. Add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black TS in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol), and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ .

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**

• **DISINTEGRATION** (701)

**Time:** 10 min, simulated gastric fluid TS being substituted for water in the test

**Acceptance criteria:** Meet the requirements

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements for *Weight Variation* with respect to alumina and to magnesia

**SPECIFIC TESTS**

• **ACID-NEUTRALIZING CAPACITY** (301): The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M)$$

- $F_A$  = theoretical acid-neutralizing capacity of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , 0.0385 mEq  
 $A$  = amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  in the specimen tested, based on the labeled quantity (mg)  
 $F_M$  = theoretical acid-neutralizing capacity of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , 0.0343 mEq  
 $M$  = amount of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$  in the specimen tested, based on the labeled quantity (mg)

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Tablets prepared with the use of Dried Aluminum Hydroxide Gel may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

## Alumina, Magnesia, and Calcium Carbonate Oral Suspension

**DEFINITION**

Alumina, Magnesia, and Calcium Carbonate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , and calcium carbonate  $(\text{CaCO}_3)$ .

**IDENTIFICATION**

• **A. IDENTIFICATION TESTS—GENERAL, Calcium** (191)

**Sample solution:** To 5 g of Oral Suspension add 25 mL of 2 N sulfuric acid, stir, and allow to stand for 5 min. Add 25 mL of alcohol, stir, and place in an ice bath for 30 min. Filter while cold, retaining the filtrate for *Identification test B*. Wash the precipitate with 50 mL of 0.75 N sulfuric acid, and discard the washings. Dissolve the precipitate in 3 N hydrochloric acid, filter, and use the filtrate.

**Acceptance criteria:** Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL, Aluminum** (191)

**Sample solution:** To the filtrate obtained in *Identification test A*, add 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, continue boiling for 2 min, and filter through hardened filter paper. (Retain the filtrate for *Identification test C*.) Wash the precipitate with 350 mL of a hot solution containing 20 mg/mL of ammonium chloride, discarding the washings. Dissolve the precipitate so obtained in 3 N hydrochloric acid.

**Acceptance criteria:** Meets the requirements

• **C. IDENTIFICATION TESTS—GENERAL, Magnesia** (191)

**Sample solution:** The filtrate obtained in *Identification test B*.

**Acceptance criteria:** Meets the requirements

**ASSAY**

• **ALUMINUM HYDROXIDE**

**Edetate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar* (0.05 M).

**Sample solution:** Transfer an amount of Oral Suspension, previously shaken in its original container, equivalent to 600 mg of aluminum hydroxide, to a tared beaker, and weigh. Add 20 mL of water, stir, and slowly add 40 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the flask, and add water to volume.

**Analysis:** Pipet 10 mL of the *Sample solution* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 3.900 mg of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

**Acceptance criteria:** 90.0%–110.0%

• **MAGNESIUM HYDROXIDE**

**Sample solution:** Prepare as directed in the Assay for Aluminum Hydroxide.

**Analysis:** Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and mix. Add 50 mL of ammonia–ammonium chloride buffer TS and 2 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mixing). Cool the solution to between 3° and 4° by immersing the beaker in an ice bath, and titrate with 0.05 M edetate disodium VS until the color changes to pure blue. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. From the volume of 0.05 M edetate disodium consumed, subtract the volume of 0.05 M edetate disodium consumed in the Assay for Calcium Carbonate. Each mL of 0.05 M



edetate disodium is equivalent to 2.916 mg of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ .

Acceptance criteria: 90.0%–110.0%

#### • CALCIUM CARBONATE

**Sample solution:** Prepare as directed in the Assay for Aluminum Hydroxide.

**Analysis:** Pipet a volume of the *Sample solution*, equivalent to 50 mg of calcium carbonate, into a 400-mL beaker, and add 200 mL of water, 5 mL of sodium hydroxide solution (1 in 2), and 250 mg of hydroxy naphthol blue. Stir with a magnetic stirrer, and titrate immediately with 0.05 M edetate disodium VS until the solution is distinctly blue. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of calcium carbonate ( $\text{CaCO}_3$ ).

Acceptance criteria: 90.0%–110.0%

#### IMPURITIES

##### • CHLORIDE AND SULFATE, Chloride (221)

**Sample solution:** Dissolve 5.0 g in 3 mL of nitric acid, add water to make 100 mL, and filter.

**Acceptance criteria:** NMT 0.14%; a 10.0-mL portion of the *Sample solution* shows no more chloride than corresponds to 1.0 mL of 0.020 N hydrochloric acid.

##### • CHLORIDE AND SULFATE, Sulfate (221)

**Sample solution:** Dissolve 5.0 g in 7 mL of 3 N hydrochloric acid, and gently heat. Cool, add water to make 250 mL, and filter.

**Acceptance criteria:** NMT 0.1%; a 20.0-mL portion of the *Sample solution* shows no more sulfate than corresponds to 0.40 mL of 0.020 N sulfuric acid.

##### • ARSENIC, Method I (211)

**Standard preparation:** Prepare as directed in *Arsenic* (211), except prepare it to contain 5  $\mu\text{g}$  of arsenic instead of 3  $\mu\text{g}$ .

**Test preparation:** Dissolve a portion of Oral Suspension, equivalent to 0.5 g of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , in 20 mL of 7 N sulfuric acid.

**Acceptance criteria:** NMT 10 ppm, based on the aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  content

#### Delete the following:

##### • HEAVY METALS (231)

**Test preparation:** Dissolve a portion of Oral Suspension, equivalent to 0.24 g of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , in 10 mL of 3 N hydrochloric acid with the aid of heat, filter, if necessary, and dilute with water to 25 mL.

**Acceptance criteria:** NMT 83 ppm, based on the aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  content. (Official 1-Jan-2018)

#### SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** Its total aerobic microbial count does not exceed  $10^2$  cfu/mL, and it meets the requirements of the test for the absence of *Escherichia coli*.

• **pH (791):** 7.5–8.5

##### • ACID-NEUTRALIZING CAPACITY (301)

**Acceptance criteria:** The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M) + 0.9 \times (F_C \times C)$$

$F_A$  = theoretical acid-neutralizing capacity of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , 0.0385 mEq

$A$  = amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  in the specimen tested, based on the labeled quantity (mg)

$F_M$  = theoretical acid-neutralizing capacity of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , 0.0343 mEq

$M$  = amount of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$  in the specimen tested, based on the labeled quantity (mg)

$F_C$  = theoretical acid-neutralizing capacity of calcium carbonate ( $\text{CaCO}_3$ ), 0.02 mEq

$C$  = amount of calcium carbonate ( $\text{CaCO}_3$ ) in the specimen tested, based on the labeled quantity (mg)

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.

• **LABELING:** Oral Suspension may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

## Alumina, Magnesia, and Calcium Carbonate Chewable Tablets

#### DEFINITION

Alumina, Magnesia, and Calcium Carbonate Chewable Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , and calcium carbonate ( $\text{CaCO}_3$ ).

#### IDENTIFICATION

##### • A. IDENTIFICATION TESTS—GENERAL, Calcium (191)

**Sample solution:** To 3 g of finely powdered Chewable Tablets add 25 mL of water and 25 mL of 2 N sulfuric acid, stir, and heat on a steam bath for 10 min. Cool, add 50 mL of alcohol, stir, and place in an ice bath for 30 min. Filter while cold, retaining the filtrate for *Identification test B*. Wash the precipitate with 50 mL of 0.75 N sulfuric acid, and discard the washings. Dissolve the precipitate in 3 N hydrochloric acid, filter, and use the filtrate.

**Acceptance criteria:** Meet the requirements

##### • B. IDENTIFICATION TESTS—GENERAL, Aluminum (191)

**Sample solution:** To the filtrate obtained in *Identification test A* add 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, continue boiling for 2 min, and filter through hardened filter paper. (Retain the filtrate for *Identification test C*.) Wash the precipitate with 350 mL of a hot solution containing 20 mg/mL of ammonium chloride, discarding the washings. Dissolve the precipitate so obtained in 3 N hydrochloric acid.

**Acceptance criteria:** Meet the requirements

##### • C. IDENTIFICATION TESTS—GENERAL, Magnesium (191)

**Sample solution:** The filtrate obtained in *Identification test B*

**Acceptance criteria:** Meet the requirements

#### ASSAY

##### • ALUMINUM HYDROXIDE

**Edetate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.

**Sample solution:** Weigh and finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent to 600 mg of aluminum hydroxide, to a beaker, add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid, with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume.

**Analysis:** Pipet 10 mL of the *Sample solution* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of



*Eдетate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Eдетate disodium titrant* consumed is equivalent to 3.900 mg of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

**Acceptance criteria:** 90.0%–110.0%

#### • MAGNESIUM HYDROXIDE

**Sample solution:** Prepare as directed in the Assay for *Aluminum Hydroxide*.

**Analysis:** Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethylamine, and mix. Add 50 mL of ammonia–ammonium chloride buffer TS and 2 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethylamine and 5 mL of dehydrated alcohol, and mixing). Cool the solution to between 3° and 4° by immersing the beaker in an ice bath, and titrate with 0.05 M edetate disodium VS until the color changes to pure blue. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. From the volume of 0.05 M edetate disodium consumed, subtract the volume of 0.05 M edetate disodium consumed in the Assay for *Calcium Carbonate*. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ .

**Acceptance criteria:** 90.0%–110.0%

#### • CALCIUM CARBONATE

**Sample solution:** Prepare as directed in the Assay for *Aluminum Hydroxide*.

**Analysis:** Pipet a volume of the *Sample solution*, equivalent to 50 mg of calcium carbonate, into a 400-mL beaker, and add 200 mL of water, 5 mL of sodium hydroxide solution (1 in 2), and 250 mg of hydroxy naphthol blue. Stir with a magnetic stirrer, and titrate immediately with 0.05 M edetate disodium VS until the solution is distinctly blue. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of calcium carbonate  $(\text{CaCO}_3)$ .

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISINTEGRATION (701)

**Time:** 45 min

**Acceptance criteria:** Meet the requirements

#### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements for *Weight Variation* with respect to aluminum hydroxide, to magnesium hydroxide, and to calcium carbonate.

### SPECIFIC TESTS

#### • ACID-NEUTRALIZING CAPACITY (301)

**Acceptance criteria:** The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M) + 0.9 \times (F_C \times C)$$

$F_A$  = theoretical acid-neutralizing capacity of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , 0.0385 mEq

$A$  = amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  in the specimen tested, based on the labeled quantity (mg)

$F_M$  = theoretical acid-neutralizing capacity of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , 0.0343 mEq

$M$  = amount of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$  in the specimen tested, based on the labeled quantity (mg)

$F_C$  = theoretical acid-neutralizing capacity of calcium carbonate  $(\text{CaCO}_3)$ , 0.02 mEq

$C$  = amount of calcium carbonate  $(\text{CaCO}_3)$  in the specimen tested, based on the labeled quantity (mg)

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** Label the Chewable Tablets to indicate that they are to be chewed before being swallowed. Chewable Tablets prepared using dried aluminum hydroxide gel may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

## Alumina, Magnesia, Calcium Carbonate, and Simethicone Chewable Tablets

### DEFINITION

Alumina, Magnesia, Calcium Carbonate, and Simethicone Chewable Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , and calcium carbonate  $(\text{CaCO}_3)$ , and an amount of polydimethylsiloxane  $([-(\text{CH}_3)_2\text{SiO}-]_n)$  that is NLT 85.0% and NMT 115.0% of the labeled amount of simethicone.

### IDENTIFICATION

#### • A. IDENTIFICATION TESTS—GENERAL, Calcium (191)

**Sample solution:** Cut a Chewable Tablet into pieces, add 50 mL of 1 N sulfuric acid, stir until the pieces disintegrate, and heat on a steam bath for 10 min. Cool, add 50 mL of alcohol, and stir. Place in an ice bath for 30 min. Filter while cold, retaining the filtrate for *Identification test B*. Wash the precipitate with 50 mL of 0.75 N sulfuric acid, and discard the washings. Dissolve the precipitate in 3 N hydrochloric acid, filter, and use the filtrate.

**Acceptance criteria:** Meet the requirements

#### • B. IDENTIFICATION TESTS—GENERAL, Aluminum (191)

**Sample solution:** To the filtrate obtained in *Identification test A*, add 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, continue boiling for 2 min, and filter through hardened filter paper. (Retain the filtrate for *Identification test C*.) Wash the precipitate with 350 mL of a hot solution containing 20 mg/mL of ammonium chloride, discarding the washings. Dissolve the precipitate so obtained in 3 N hydrochloric acid.

**Acceptance criteria:** Meet the requirements

#### • C. IDENTIFICATION TESTS—GENERAL, Magnesium (191)

**Sample solution:** The filtrate obtained in *Identification test B*

**Acceptance criteria:** Meet the requirements

#### • D. INFRARED ABSORPTION (197S)

**Sample solution:** Prepare as directed in the Assay for *Polydimethylsiloxane*.

**Analysis:** Proceed as directed using a 0.5-mm cell.

**Acceptance criteria:** Meet the requirements

### ASSAY

#### • ALUMINUM HYDROXIDE

**Eдетate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Eдетate Disodium, Twentieth-Molar (0.05 M)*.



**Sample solution:** Transfer a number of Chewable Tablets, equivalent of about 665 mg of aluminum hydroxide, to a suitable beaker. Add 15 mL of hydrochloric acid, and swirl to dissolve the Chewable Tablets. Add 80 mL of water, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.

**Analysis:** Pipet 20 mL of the *Sample solution* into a 250-mL beaker, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 20 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

**Acceptance criteria:** 90.0%–110.0%

#### • MAGNESIUM HYDROXIDE

**Lanthanum chloride solution:** Transfer 17.6 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, and carefully add 50 mL of hydrochloric acid. Allow to cool, and dilute with water to volume.

**Dilute hydrochloric acid:** Dilute 226 mL of hydrochloric acid to 1000 mL with water.

**Potassium chloride solution:** 30 mg/mL in water

**Magnesium stock solution:** Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water, slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with water to volume. Transfer 1.0 mL of this solution to a 100-mL volumetric flask to obtain a solution containing 10 µg/mL of magnesium (Mg).

**Standard solutions:** To three separate 100-mL volumetric flasks each containing 5.0 mL of *Lanthanum chloride solution*, add 1.0, 2.0, and 3.0 mL, respectively, of the *Magnesium stock solution*. Dilute each with water to volume. These solutions contain 0.1, 0.2, and 0.3 µg/mL of magnesium (Mg), respectively.

**Sample stock solution:** Transfer a number of Chewable Tablets, equivalent of 250 mg of magnesium hydroxide (100 mg of magnesium), to a 1000-mL volumetric flask. Add 500 mL of *Dilute hydrochloric acid*, and swirl to dissolve the Chewable Tablets. Add 100.0 mL of *Potassium chloride solution*, and dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 2.0 mL of *Sample stock solution* to a second 100-mL volumetric flask, add 5.0 mL of *Lanthanum chloride solution*, and dilute with water to volume.

**Blank:** Add 50 mL of *Dilute hydrochloric acid* and 10.0 mL of *Potassium chloride solution* to a 100-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, and dilute with water to volume. Transfer 2.0 mL of this solution to a third 100-mL volumetric flask, add 5.0 mL of *Lanthanum chloride solution*, and dilute with water to volume.

**Analysis:** Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a magnesium hollow-cathode lamp and an air–acetylene flame, using the *Blank* to set the instrument. Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of magnesium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration,  $C$ , in µg/mL, of magnesium in the *Sample solution*.

Calculate the percentage of the labeled amount of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$  in the portion of Tablets taken:

$$\text{Result} = (C/C_u) \times (M_r/A_r) \times 100$$

$C$  = concentration of magnesium in the *Sample solution*, as determined above (µg/mL)

$C_u$  = nominal concentration of magnesium hydroxide in the *Sample solution* (µg/mL)

$M_r$  = molecular weight of magnesium hydroxide, 58.34

$A_r$  = atomic weight of magnesium, 24.305

**Acceptance criteria:** 90.0%–110.0%

#### • CALCIUM CARBONATE

**Sample solution:** Transfer a number of Chewable Tablets, equivalent of about 665 mg of aluminum hydroxide, to a suitable beaker. Add 15 mL of hydrochloric acid, and swirl to dissolve the Chewable Tablets. Add 80 mL of water, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.

**Analysis:** Pipet a volume of the *Sample solution*, equivalent to 50 mg of calcium carbonate, into a 400-mL beaker, and add 200 mL of water, a volume of sodium hydroxide solution (1 in 2) equivalent to the volume of the *Sample solution* taken, and 250 mg of hydroxy naphthol blue. Stir with a magnetic stirrer, and titrate immediately with 0.05 M edetate disodium VS until the solution is distinctly blue. Perform a blank determination, substituting a volume of water equivalent to the volume of the *Sample solution* taken, and make any necessary correction. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of calcium carbonate ( $\text{CaCO}_3$ ).

**Acceptance criteria:** 90.0%–110.0%

#### • POLYDIMETHYLSILOXANE

**Dilute hydrochloric acid:** Dilute 400 mL of hydrochloric acid with sufficient water to make 1000 mL.

**Standard solution:** Transfer 60 mg of USP

Polydimethylsiloxane RS to a separator, add 30.0 mL of chloroform and 60 mL of *Dilute hydrochloric acid*, shake for 30 s, and allow the phases to separate. Remove 10 mL of the lower, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture to obtain a clear supernatant.

**Sample solution:** Weigh and finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent of 60 mg of simethicone, to a suitable screw-capped bottle. Add 30.0 mL of chloroform and 60 mL of *Dilute hydrochloric acid*, and allow to stand, with frequent shaking, until the Chewable Tablets are dissolved. Transfer the contents of the bottle to a separator, shake, and allow the phases to separate. Remove 10 mL of the lower, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture to obtain a clear supernatant.

**Blank:** Place 30.0 mL of chloroform and 60 mL of *Dilute hydrochloric acid* in a separator, shake for 30 s, and allow the phases to separate. Remove 10 mL of the lower, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap that has an inert liner, agitate vigorously, and centrifuge the mixture to obtain a clear supernatant.

**Analysis:** Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution* in 0.5-mm cells at the wavelength of maximum absorbance at about 7.9 µm, with a suitable IR spectrophotometer, using the *Blank* to set the instrument.



Calculate the percentage of polydimethylsiloxane  $[(\text{CH}_3)_2\text{SiO}]_n$  in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (W_S/W_U) \times 100$$

- $A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $W_S$  = weight of USP Polydimethylsiloxane RS taken to prepare the *Standard solution* (mg)  
 $W_U$  = nominal amount of simethicone in the portion of the Tablets taken to prepare the *Sample solution* (mg)

Acceptance criteria: 85.0%–115.0%

## PERFORMANCE TESTS

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation* with respect to aluminum hydroxide, to magnesium hydroxide, and to calcium carbonate.

## SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count is NMT  $2 \times 10^2$  cfu/g, the total combined molds and yeasts count is NMT  $2 \times 10^2$  cfu/g, and the Chewable Tablets meet the requirements of the test for the absence of *Salmonella* species and *Escherichia coli*.

## ACID-NEUTRALIZING CAPACITY (301)

**Sample solution:** Dissolve an accurately counted number of Chewable Tablets, equivalent to about 120 mEq of acid-neutralizing capacity, in 400 mL of water. Transfer the mixture to a 500-mL volumetric flask, and dilute with water to volume.

**Analysis:** Proceed as directed in the section *Procedure for Powders, Effervescent Solids, Suspensions and Other Liquids, Lozenges Nonchewable Tablets, Chewable Tablets, and Capsules* using 75.0 mL of the *Sample solution*.

**Acceptance criteria:** The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M) + 0.9 \times (F_C \times C)$$

- $F_A$  = theoretical acid-neutralizing capacity of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , 0.0385 mEq  
 $A$  = amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  in the specimen tested, based on the labeled quantity (mg)  
 $F_M$  = theoretical acid-neutralizing capacity of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , 0.0343 mEq  
 $M$  = amount of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$  in the specimen tested, based on the labeled quantity (mg)  
 $F_C$  = theoretical acid-neutralizing capacity of calcium carbonate  $(\text{CaCO}_3)$ , 0.02 mEq  
 $C$  = amount of calcium carbonate  $(\text{CaCO}_3)$  in the specimen tested, based on the labeled quantity (mg)

## SODIUM CONTENT

**Potassium chloride solution:** 30 mg/mL of potassium chloride

**Dilute hydrochloric acid:** Dilute 226 mL of hydrochloric acid with sufficient water to make 1000 mL.

**Standard stock solution:** Transfer 2.5420 g of sodium chloride, previously dried at  $105^\circ$  for 2 h, to a 1000-mL volumetric flask, and dissolve in and dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, and dilute with water to volume.

**Standard solutions:** To three separate 100-mL volumetric flasks, each containing 10.0 mL of *Potassium chloride*

*solution* and 3.0 mL of *Dilute hydrochloric acid*, add 10.0, 20.0, and 30.0 mL, respectively, of the *Standard stock solution*. The resulting *Standard solutions* contain 1.0, 2.0, and 3.0  $\mu\text{g/mL}$  of sodium (Na), respectively.

**Sample stock solution:** Weigh 10 Chewable Tablets, and determine the average weight,  $A$ , in mg. Cut 4 Chewable Tablets into pieces, combine the pieces, and weigh them. Transfer the combined pieces to a 500-mL volumetric flask, add 150 mL of *Dilute hydrochloric acid*, and swirl gently to dissolve the pieces. Dilute with water to volume.

**Sample solution:** Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, add 10.0 mL of *Potassium chloride solution*, and dilute with water to volume.

**Blank solution:** Combine 3.0 mL of *Dilute hydrochloric acid* and 10.0 mL of *Potassium chloride solution* in a 100-mL volumetric flask, and dilute with water to volume.

## Analysis

**Samples:** *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a sodium hollow-cathode lamp and an air-acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard solutions* versus concentration, in  $\mu\text{g/mL}$ , of sodium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration,  $C$ , in  $\mu\text{g/mL}$ , of sodium in the *Sample solution*.

Calculate the quantity, in mg, of sodium (Na) in each Chewable Tablet taken:

$$\text{Result} = (A/W) \times C \times D \times F$$

- $A$  = average weight of each Tablet (mg)  
 $W$  = weight of the portion of Chewable Tablets taken to prepare the *Sample solution* (mg)  
 $C$  = concentration of sodium in the *Sample solution* ( $\mu\text{g/mL}$ )  
 $D$  = dilution factor for the *Sample solution*, 5000  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

**Acceptance criteria:** Chewable Tablets contain NMT 5 mg/Tablet of sodium, except when labeled as containing more than 5 mg/Tablet of sodium; then they contain NMT 110% of the labeled amount.

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers.
- LABELING:** The labeling indicates that the Chewable Tablets are to be chewed before swallowing. Label the Chewable Tablets to state the sodium content, if it is greater than 5 mg/Chewable Tablet.
- USP REFERENCE STANDARDS (11)**  
USP Polydimethylsiloxane RS

## Alumina, Magnesia, and Simethicone Oral Suspension

### DEFINITION

Alumina, Magnesia, and Simethicone Oral Suspension contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , and an amount of polydimethylsiloxane  $[(\text{CH}_3)_2\text{SiO}]_n$  that is NLT 85.0% and NMT 115.0% of the labeled amount of simethicone.



**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197S)

**Sample solution:** Prepare as directed in the Assay for Polydimethylsiloxane.

**Analysis:** Proceed as directed using a 0.5-mm cell.

**Acceptance criteria:** Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL, Magnesium** (191)

**Sample solution:** Add 5 g of Oral Suspension to 10 mL of 3 N hydrochloric acid, then add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution just changes to deep yellow, then continue boiling for 2 min, and filter.

**Acceptance criteria:** Meets the requirements

• **C. IDENTIFICATION TESTS—GENERAL, Aluminum** (191)

**Sample solution:** Wash the precipitate from Identification test B with hot ammonium chloride solution (1 in 50), and dissolve the precipitate in hydrochloric acid. Divide this solution into two portions.

**Analysis 1:** Add, dropwise, 6 N ammonium hydroxide to one portion of the *Sample solution*.

**Acceptance criteria 1:** A gelatinous white precipitate, which does not dissolve in an excess of 6 N ammonium hydroxide, is obtained.

**Analysis 2:** Add, dropwise, 1 N sodium hydroxide to the second portion of the *Sample solution*.

**Acceptance criteria 2:** A gelatinous white precipitate, which dissolves in an excess of 1 N sodium hydroxide, leaving some turbidity, is obtained.

**ASSAY**• **ALUMINUM HYDROXIDE**

**Edetate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar* (0.05 M).

**Sample solution:** Transfer a measured amount of Oral Suspension, previously well shaken in its original container, equivalent to 800 mg of aluminum hydroxide, to a suitable beaker. Add 20 mL of water, stir, and slowly add 10 mL of hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.

**Analysis:** Pipet 10 mL of the *Sample solution* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 3.900 mg of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

**Acceptance criteria:** 90.0%–115.0%

• **MAGNESIUM HYDROXIDE**

**Sample solution:** Prepare as directed in the Assay for Aluminum Hydroxide.

**Analysis:** Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mixing). Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting water for the *Sample solution*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ .

**Acceptance criteria:** 90.0%–115.0%

• **POLYDIMETHYLSILOXANE**

**Standard solution:** Prepare similarly to the *Sample solution* below, except dissolve 50 mg of USP Polydimethylsiloxane RS in 25.0 mL of toluene, add 40 mL of 0.1 N sodium hydroxide, and add a volume of water equal to that of the specimen of Oral Suspension taken for the *Sample solution*.

**Sample solution:** Transfer an amount of Oral Suspension, equivalent to 50 mg of simethicone, to a suitable round, narrow-mouth, screw-capped, 120-mL bottle, add 40 mL of 0.1 N sodium hydroxide, and swirl to disperse. Add 25.0 mL of toluene, close the bottle securely with a cap having an inert liner, and shake for 15 min on a reciprocating shaker (e.g., about 200 oscillations/min and a stroke of  $38 \pm 2$  mm). Transfer the mixture to a 125-mL separator. Remove about 5 mL of the upper, organic layer to a screw-capped, 15-mL test tube containing 0.5 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.

**Blank:** Mix 10 mL of toluene with 0.5 g of anhydrous sodium sulfate, and centrifuge to obtain a clear supernatant.

**Analysis:** Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution* in 0.5-mm cells at the wavelength of maximum absorbance at about 7.9  $\mu\text{m}$ , with a suitable IR spectrophotometer, using the *Blank* to set the instrument.

Calculate the percentage of polydimethylsiloxane  $[(\text{--}(\text{CH}_3)_2\text{SiO--})_n]$  in the portion of Oral Suspension taken:

$$\text{Result} = (A_U/A_S) \times (W_S/W_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$W_S$  = weight of USP Polydimethylsiloxane RS taken to prepare the *Standard solution* (mg)

$W_U$  = nominal amount of simethicone in the portion of the Oral Suspension taken to prepare the *Sample solution* (mg)

**Acceptance criteria:** 85.0%–115.0%

**SPECIFIC TESTS**

• **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count is NMT  $10^2$  cfu/g, and it meets the requirements of the test for the absence of *Escherichia coli*.

• **ACID-NEUTRALIZING CAPACITY** (301)

**Acceptance criteria:** The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M)$$

$F_A$  = theoretical acid-neutralizing capacity of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , 0.0385 mEq

$A$  = amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  in the specimen tested, based on the labeled quantity (mg)

$F_M$  = theoretical acid-neutralizing capacity of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , 0.0343 mEq

$M$  = amount of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$  in the specimen tested, based on the labeled quantity (mg)

• **PH** (791): 7.0–8.6• **SODIUM CONTENT**

**Potassium chloride solution:** 38 mg/mL of potassium chloride



**Sodium chloride stock solution:** 25.42 µg/mL of sodium chloride (previously dried at 105° for 2 h) in water. The solution contains 10 µg/mL of sodium.

**Standard solutions:** On the day of use, transfer 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* to each of two 100-mL volumetric flasks. To the respective flasks add 5.0 and 10.0 mL of *Sodium chloride stock solution*. Dilute with water to volume. The resulting *Standard solutions* contain 0.5 and 1.0 µg/mL of sodium (Na), respectively.

**Sample solution:** Transfer 5.0 mL of Oral Suspension, previously well shaken in its original container, to a 100-mL volumetric flask. Add 50 mL of 1 N hydrochloric acid, boil for 15 min, cool to room temperature, and dilute with water to volume. Filter, discarding the first few mL of the filtrate. Transfer 5.0 mL of the filtrate to a 100-mL volumetric flask containing 10.0 mL of *Potassium chloride solution*, and dilute with water to volume.

**Blank solution:** Combine 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* in a 100-mL volumetric flask, and dilute with water to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a sodium hollow-cathode lamp and an air-acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of sodium, and draw a straight line between the plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL, of sodium in the *Sample solution*.

Calculate the quantity, in mg, of sodium (Na) in each mL of Oral Suspension taken:

$$\text{Result} = (1/N) \times C \times D \times F$$

- N* = volume of the Oral Suspension taken to prepare the *Sample solution*, 5.0 mL  
*C* = concentration of sodium in the *Sample solution* (µg/mL)  
*D* = dilution factor for the *Sample solution*, 2000  
*F* = conversion factor, 0.001 mg/µg

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.
- **LABELING:** Oral Suspension may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide [Al(OH)<sub>3</sub>]. Label it to state the sodium content if it is greater than 1 mg/mL.
- **USP REFERENCE STANDARDS (11)**  
 USP Polydimethylsiloxane RS

## Alumina, Magnesia, and Simethicone Chewable Tablets

#### DEFINITION

Alumina, Magnesia, and Simethicone Chewable Tablets contain the equivalent of NLT 90.0% and NMT 115.0% of the labeled amounts of aluminum hydroxide [Al(OH)<sub>3</sub>] and magnesium hydroxide [Mg(OH)<sub>2</sub>], and an amount of polydimethylsiloxane [(—(CH<sub>3</sub>)<sub>2</sub>SiO—)<sub>n</sub>] that is NLT 85.0% and NMT 115.0% of the labeled amount of simethicone.

#### IDENTIFICATION

##### • A. INFRARED ABSORPTION (197S)

**Sample solution:** Prepare as directed in the Assay for *Polydimethylsiloxane*.

**Analysis:** Proceed as directed using a 0.5-mm cell.

**Acceptance criteria:** Meet the requirements

##### • B. IDENTIFICATION TESTS—GENERAL, Magnesium (191)

**Sample solution:** To a portion of finely powdered Chewable Tablets, equivalent to 600 mg of magnesium hydroxide, add 25 mL of 3 N hydrochloric acid and 25 mL of water, and mix. Boil gently for 2 min. Allow to cool, and filter. Add 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution just turns to deep yellow. Continue boiling for 2 min, and filter.

**Acceptance criteria:** Meet the requirements

##### • C. IDENTIFICATION TESTS—GENERAL, Aluminum (191)

**Sample solution:** Wash the precipitate from *Identification test B* with hot ammonium chloride solution (1 in 50), and dissolve the precipitate in hydrochloric acid. Divide this solution into two portions.

**Analysis 1:** Add, dropwise, 6 N ammonium hydroxide to one portion of the *Sample solution*.

**Acceptance criteria 1:** A gelatinous white precipitate, which does not dissolve in an excess of 6 N ammonium hydroxide, is obtained.

**Analysis 2:** Add, dropwise, 1 N sodium hydroxide to the second portion of the *Sample solution*.

**Acceptance criteria 2:** A gelatinous white precipitate, which dissolves in an excess of 1 N sodium hydroxide, leaving some turbidity, is obtained.

#### ASSAY

##### • ALUMINUM HYDROXIDE

**Edetate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.

**Sample solution:** Weigh and finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent of 800 mg of aluminum hydroxide, to a 150-mL beaker. Add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool to room temperature, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, and add water to volume.

**Analysis:** Pipet 10 mL of the *Sample solution* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the excess edetate disodium with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 3.900 mg of aluminum hydroxide [Al(OH)<sub>3</sub>].

**Acceptance criteria:** 90.0%–115.0%

##### • MAGNESIUM HYDROXIDE

**Sample solution:** Prepare as directed in the Assay for *Aluminum Hydroxide*.

**Analysis:** Pipet a volume of the *Sample solution*, equivalent to 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mixing). Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting water for the *Sample solution*, and make any necessary correction.



Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ .

Acceptance criteria: 90.0%–115.0%

#### • POLYDIMETHYLSILOXANE

**Standard solution:** Prepare similarly to the *Sample solution* below, using 33 mg of USP Polydimethylsiloxane RS.

**Sample solution:** Weigh and finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent of 33 mg of simethicone, to a suitable round, narrow-mouth, screw-capped, 120-mL bottle. Add 40 mL of 0.1 N sodium hydroxide, and swirl to disperse. Add 20.0 mL of toluene, close the bottle securely with a cap having an inert liner, and shake for 30 min on a reciprocating shaker (e.g., 200 oscillations/min and a stroke of  $38 \pm 2$  mm). Transfer the mixture to a 125-mL separator, and allow to separate. Remove the upper, organic layer to a screw-capped, centrifuge tube containing 2 g of anhydrous sodium sulfate. Close the tube with a screw-cap having an inert liner, agitate vigorously, and centrifuge the mixture until a clear supernatant is obtained.

**Blank:** Mix 10 mL of toluene with 1 g of anhydrous sodium sulfate, and centrifuge to obtain a clear supernatant.

**Analysis:** Concomitantly determine the absorbances of the *Standard solution* and *Sample solution* in 0.5-mm cells at the wavelength of maximum absorbance at about  $7.9 \mu\text{m}$  ( $1265.8 \text{ cm}^{-1}$ ), with a suitable IR spectrophotometer, using the *Blank* to set the instrument. Calculate the percentage of polydimethylsiloxane  $[(\text{--}(\text{CH}_3)_2\text{SiO--})_n]$  in the portion of Chewable Tablets taken:

$$\text{Result} = (A_U/A_S) \times (W_S/W_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$W_S$  = weight of USP Polydimethylsiloxane RS taken to prepare the *Standard solution* (mg)

$W_U$  = nominal amount of simethicone in the portion of the Chewable Tablets taken to prepare the *Sample solution* (mg)

Acceptance criteria: 85.0%–115.0%

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium hydroxide

#### SPECIFIC TESTS

- **ACID-NEUTRALIZING CAPACITY (301)**

Acceptance criteria: The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq, and NLT the number of mEq calculated by the formula:

$$\text{Result} = 0.55 \times (F_A \times A) + 0.8 \times (F_M \times M)$$

$F_A$  = theoretical acid-neutralizing capacity of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , 0.0385 mEq

$A$  = amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  in the specimen tested, based on the labeled quantity (mg)

$F_M$  = theoretical acid-neutralizing capacity of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ , 0.0343 mEq

$M$  = amount of magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$  in the specimen tested, based on the labeled quantity (mg)

- **SODIUM CONTENT**

Potassium chloride solution: 38 mg/mL of potassium chloride

**Sodium chloride stock solution:** 25.42  $\mu\text{g/mL}$  of sodium chloride (previously dried at  $105^\circ$  for 2 h) in water. The solution contains 10  $\mu\text{g/mL}$  of sodium.

**Standard solutions:** On the day of use, transfer 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* to each of two 100-mL volumetric flasks. To the respective flasks add 5.0 and 10.0 mL of *Sodium chloride stock solution*. Dilute with water to volume. The resulting *Standard solutions* contain 0.5 and 1.0  $\mu\text{g/mL}$  of sodium (Na), respectively.

**Sample solution:** Weigh and finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent to the average weight of 1 Chewable Tablet, to a 100-mL volumetric flask. Add 50 mL of 1 N hydrochloric acid, boil for 15 min, cool to room temperature, and dilute with water to volume. Filter, discarding the first few mL of the filtrate. Transfer 5.0 mL of the filtrate to a 100-mL volumetric flask containing 10.0 mL of *Potassium chloride solution*, and dilute with water to volume.

**Blank solution:** Combine 4.0 mL of 1 N hydrochloric acid and 10.0 mL of *Potassium chloride solution* in a 100-mL volumetric flask, and dilute with water to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a sodium hollow-cathode lamp and an air-acetylene flame, using the *Blank solution* as the blank. Plot the absorbances of the *Standard solutions* versus concentration, in  $\mu\text{g/mL}$ , of sodium, and draw a straight line between the plotted points. From the graph so obtained, determine the concentration,  $C$ , in  $\mu\text{g/mL}$ , of sodium in the *Sample solution*.

Calculate the quantity, in mg, of sodium (Na) in each Chewable Tablet taken:

$$\text{Result} = C \times D \times F$$

$C$  = concentration of sodium in the *Sample solution* ( $\mu\text{g/mL}$ )

$D$  = dilution factor for the *Sample solution*, 2000

$F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label the Chewable Tablets to indicate that they are to be chewed before being swallowed. Label the Chewable Tablets to state the sodium content if it is greater than 5 mg/Tablet. The Chewable Tablets may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .
- **USP REFERENCE STANDARDS (11)**  
USP Polydimethylsiloxane RS

### Alumina and Magnesium Carbonate Oral Suspension

» Alumina and Magnesium Carbonate Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of



the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium carbonate ( $\text{MgCO}_3$ ).

**Packaging and storage**—Preserve in tight containers, and avoid freezing.

**Identification**—

**A:** Place about 1 g in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

**B:** To a solution of 5 g in 10 mL of 3 N hydrochloric acid add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* (191).

**C:** Wash the precipitate obtained in *Identification* test B with a hot solution of ammonium chloride (1 in 50), and dissolve the precipitate in hydrochloric acid: the solution responds to the tests for *Aluminum* (191).

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for absence of *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

**Acid-neutralizing capacity (301)**—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than the number of mEq calculated by the formula:

$$0.55(0.0385A) + 0.8(0.024M)$$

in which 0.0385 and 0.024 are the theoretical acid-neutralizing capacities, in mEq, of  $\text{Al}(\text{OH})_3$  and  $\text{MgCO}_3$ , respectively; and A and M are the respective quantities, in mg, of  $\text{Al}(\text{OH})_3$  and  $\text{MgCO}_3$  in the specimen tested, based on the labeled quantities.

**pH (791):** between 7.5 and 9.5.

**Assay for aluminum hydroxide**—

**Potassium chloride solution**—Prepare a solution containing 4.5 g of potassium chloride in each 100 mL.

**Aluminum stock solution**—Transfer 1.000 g of aluminum wire to a 1000-mL volumetric flask, and add 50 mL of 6 N hydrochloric acid. Swirl to ensure contact of the aluminum and the acid, and allow the reaction to proceed until all of the aluminum has dissolved. Dilute with water to volume, and mix.

**Standard preparations**—To separate 100-mL volumetric flasks, each containing 10 mL of *Potassium chloride solution*, transfer 9.0, 10.0, and 11.0 mL, respectively, of *Aluminum stock solution*, dilute with water to volume, and mix. These *Standard preparations* contain 90.0, 100.0, and 110.0  $\mu\text{g}$  of aluminum per mL, respectively.

**Assay preparation**—Transfer an accurately measured quantity of Oral Suspension, previously shaken in its original container, equivalent to about 75 mg of aluminum hydroxide, to a suitable beaker. Add 25 mL of 6 N hydrochloric acid, and heat on a steam bath for 30 minutes, with occasional swirling. Cool, and transfer with the aid of water to a 250-mL volumetric flask containing 25 mL of *Potassium chloride solution*. Dilute with water to volume, mix, and filter.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the aluminum emission line at 309.3 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with an aluminum hollow-cathode lamp and a nitrous oxide-acetylene flame, using water as

the blank. Calculate the quantity, in mg, of  $\text{Al}(\text{OH})_3$  in the portion of Oral Suspension taken by the formula:

$$(78.00 / 26.98)(0.25)(A_U / R_S)$$

in which 78.00 is the molecular weight of aluminum hydroxide; 26.98 is the atomic weight of aluminum;  $A_U$  is the absorbance of the *Assay preparation*; and  $R_S$  is the average of the ratios of the absorbances of the *Standard preparations* to their respective concentrations, in  $\mu\text{g}$  of aluminum per mL.

**Assay for magnesium carbonate**—

**Lanthanum chloride solution**—Prepare a solution of lanthanum chloride in water containing 5 mg per mL.

**Magnesium stock solution**—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 50 mL of water, and slowly add 10 mL of hydrochloric acid. Dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Standard preparations**—To separate 100-mL volumetric flasks, each containing 10 mL of *Lanthanum chloride solution*, transfer 1.70 mL and 1.80 mL, respectively, of *Magnesium stock solution*, dilute with water to volume, and mix. These *Standard preparations* contain 1.7  $\mu\text{g}$  of magnesium per mL and 1.8  $\mu\text{g}$  of magnesium per mL, respectively.

**Assay preparation**—Quantitatively dilute an accurately measured volume of the *Assay preparation* prepared as directed in the *Assay for aluminum hydroxide* with water to obtain a solution having a concentration of about 6  $\mu\text{g}$  of magnesium carbonate per mL.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a magnesium hollow-cathode lamp and an air-acetylene flame, using water as the blank. Calculate the quantity, in mg, of magnesium carbonate ( $\text{MgCO}_3$ ) in the portion of Oral Suspension taken by the formula:

$$(84.31 / 24.31)(L / D)(A_U / R_S)$$

in which 84.31 is the molecular weight of magnesium carbonate; 24.31 is the atomic weight of magnesium; L is the labeled quantity, in mg, of magnesium carbonate in the portion of Oral Suspension taken; D is the concentration, in  $\mu\text{g}$  of magnesium carbonate per mL, of the *Assay preparation*, based on the labeled amount of magnesium carbonate in the portion of Oral Suspension taken and the extent of dilution;  $A_U$  is the absorbance of the *Assay preparation*; and  $R_S$  is the average of the ratios of the absorbances of the *Standard preparations* to their respective concentrations, in  $\mu\text{g}$  of magnesium per mL.

## Alumina and Magnesium Carbonate Tablets

» Alumina and Magnesium Carbonate Tablets contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium carbonate ( $\text{MgCO}_3$ ).

**Packaging and storage**—Preserve in tight containers.

**Identification**—

**A:** Place about 1 g of finely powdered Tablets in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately



insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

**B:** To a 7-g portion of finely powdered Tablets add 10 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution changes to deep yellow. Continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* (191).

**C:** Wash the precipitate obtained in *Identification* test B with a hot solution of ammonium chloride (1 in 50), and dissolve the precipitate in hydrochloric acid: the solution responds to the tests for *Aluminum* (191).

**Disintegration** (701): 10 minutes, simulated gastric fluid TS being substituted for water in the test.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium carbonate.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

#### Assay for aluminum hydroxide—

**Potassium chloride solution**—Prepare a solution containing 38.1 g of potassium chloride in each 1000 mL.

**Digestion fluid**—Mix 5 mL of hydrochloric acid, 10 mL of nitric acid, and 10 mL of water, and use promptly.

**Aluminum stock solution**—Transfer 1.000 g of aluminum metal to a 1000-mL volumetric flask, and add 50 mL of 6 N hydrochloric acid. Swirl to ensure contact of the aluminum and the acid, and allow the reaction to proceed until all of the aluminum has dissolved. Dilute with water to volume, and mix.

**Standard preparations**—To separate 100-mL volumetric flasks transfer 3.0 mL, 4.0 mL, and 5.0 mL of *Aluminum stock solution*, respectively. To each flask add 10 mL of *Potassium chloride solution* and 7.5 mL of *Digestion fluid*, dilute with water to volume, and mix. These *Standard preparations* contain 30, 40, and 50 µg of aluminum per mL, respectively.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 30 mg of aluminum hydroxide, to a 100-mL volumetric flask, add 25-mL of *Digestion fluid*, and heat on a steam bath for 30 minutes or on a hot plate until the volume is reduced by about one-half. Cool, dilute with water to volume, and mix. Filter, discarding the first 20 mL of the filtrate. Transfer 15.0 mL of the filtrate to a 50-mL volumetric flask, add 5.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix. [NOTE—Reserve a portion of the filtrate for use in the *Assay for magnesium carbonate*.]

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the aluminum emission line at 309.3 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with an aluminum hollow-cathode lamp and a nitrous oxide-acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of aluminum, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C, in µg per mL, of aluminum in each mL of the *Assay preparation*. Calculate the quantity, in mg, of aluminum hydroxide [Al(OH)<sub>3</sub>] in the portion of Tablets taken by the formula:

$$(78.00 / 26.98)(C / 3)$$

in which 78.00 is the molecular weight of aluminum hydroxide, and 26.98 is the atomic weight of aluminum.

#### Assay for magnesium carbonate—

**Lanthanum chloride solution**—Transfer 17.6 g of lanthanum chloride to a 1000-mL volumetric flask, add 500 mL of water, and carefully add 50 mL of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume, and mix.

**Digestion fluid**—Mix 5 mL of hydrochloric acid, 10 mL of nitric acid, and 10 mL of water, and use promptly.

**Magnesium stock solution**—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 50 mL of water, and slowly add 10 mL of hydrochloric acid. Dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

**Standard preparations**—To separate 100-mL volumetric flasks transfer 4.0, 6.0, and 8.0 mL of *Magnesium stock solution*, respectively. To each flask add 0.5 mL of *Digestion fluid* and 10 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix. These *Standard preparations* contain 0.40, 0.60, and 0.80 µg of magnesium per mL, respectively.

**Assay preparation**—Transfer an accurately measured volume of the filtrate used to prepare the *Assay preparation* in the *Assay for aluminum hydroxide*, equivalent to about 0.4 mg of magnesium carbonate, to a 200-mL volumetric flask, add 20 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a magnesium hollow-cathode lamp and an air-acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of magnesium, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, C, in µg per mL, of magnesium in each mL of the *Assay preparation*. Calculate the quantity, in mg, of magnesium carbonate (MgCO<sub>3</sub>) in the portion of Tablets taken by the formula:

$$(84.31 / 24.31)(20C / V)$$

in which 84.31 is the molecular weight of magnesium carbonate; 24.31 is the atomic weight of magnesium; and V is the volume taken, in mL, of the *Assay preparation* prepared as directed in the *Assay for aluminum hydroxide*.

## Alumina, Magnesium Carbonate, and Magnesium Oxide Tablets

» Alumina, Magnesium Carbonate, and Magnesium Oxide Tablets contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide [Al(OH)<sub>3</sub>] and magnesium carbonate (MgCO<sub>3</sub>), and not less than 85.0 percent and not more than 115.0 percent of the labeled amount of magnesium oxide (MgO).

**Packaging and storage**—Preserve in tight containers.

#### Identification—

**A:** Place about 3 g of finely powdered Tablets in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

**B:** To the solution in the flask obtained in *Identification* test A add 5 drops of methyl red TS, and heat to boiling. Add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, continue boiling for 2 minutes, and filter through hardened filter paper. (Retain the filtrate for *Identification* test C.) Wash the precipitate with 350 mL



of a hot ammonium chloride solution (1 in 50), discarding the washings: the precipitate so obtained, dissolved in 3 N hydrochloric acid, responds to the tests for *Aluminum* (191).

C: The filtrate obtained in *Identification* test B responds to the tests for *Magnesium* (191).

**Disintegration** (701): 10 minutes, simulated gastric fluid TS being substituted for water in the test.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to alumina, to magnesium carbonate, and to magnesium oxide.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

#### Assay for aluminum hydroxide—

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1200 mg of aluminum hydroxide, to a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, add water to volume, and mix.

*Procedure*—Pipet 10 mL of the *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat near the boiling point for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

**Assay for magnesium carbonate**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 750 mg of magnesium carbonate, to a 250-mL conical flask fitted with a two-hole stopper. Fill the lower transverse section of a U-shaped drying tube of about 15-mm internal diameter and 15-cm height with loosely packed glass wool. Place in one arm of the tube about 5 g of anhydrous calcium chloride, and accurately weigh the tube and the contents. Into the other arm of the tube place 9.5 g to 10.5 g of soda lime, and again weigh accurately. Insert stoppers in the open arms of the U-tube, and connect the side tube of the arm filled with soda lime to a calcium chloride drying tube, which in turn is connected to one of the holes in the stopper of the 250-mL conical flask. Attach a dropping funnel to the other hole in the stopper of the 250-mL conical flask. Add 100 mL of water and 10 mL of a mixture of hydrochloric acid and nitric acid (4:1) to the 250-mL conical flask through the dropping funnel, and close the dropping funnel. Heat the 250-mL conical flask at 95° for 1 hour, and allow the evolved carbon dioxide to pass through the U-tube. Replace the dropping funnel with a source of carbon dioxide-free air, and pass the carbon dioxide-free air through the apparatus at a rate of about 75 mL per minute for 30 minutes. Disconnect the U-tube, cool to room temperature, remove the stoppers, and weigh. The increase in weight corresponds to the quantity of carbon dioxide evolved. Calculate the quantity, in mg, of magnesium carbonate in each Tablet taken by the formula:

$$(84.31 / 44.01)(I)(W_A / W_P)$$

in which 84.31 and 44.01 are the molecular weights of magnesium carbonate and carbon dioxide, respectively; *I* is the quantity, in mg, of carbon dioxide evolved from the portion of Tablets taken;  $W_A$  is the average weight, in g, of 1

Tablet; and  $W_P$  is the weight, in g, of the portion of Tablets taken.

**Assay for magnesium oxide**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1000 mg of magnesium carbonate and magnesium oxide combined, to a beaker, add 20 mL of water, and slowly add 40 mL of 3 N hydrochloric acid, with mixing. Heat the mixture to boiling, cool, and filter into a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the filter. Add water to volume, and mix. Transfer 20.0 mL of this solution to a 400-mL beaker, add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia–ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the assay solution, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 1.216 mg of Mg. Calculate the quantity, in mg, of magnesium equivalent in each Tablet taken by the formula:

$$10T(W_A / W_P)$$

in which *T* is the magnesium equivalent obtained in the titration;  $W_A$  is the average weight, in g, of 1 Tablet; and  $W_P$  is the weight, in g, of the portion of Tablets taken. Calculate the quantity, in mg, of magnesium oxide in each Tablet taken by the formula:

$$(40.30 / 24.31)(A - 0.2883B)$$

in which 40.30 and 24.31 are the molecular weight of magnesium oxide and the atomic weight of magnesium, respectively; *A* is the quantity, in mg, of magnesium equivalent in each Tablet; and *B* is the quantity, in mg, of magnesium carbonate in each Tablet, as determined in the *Assay for magnesium carbonate*.

## Alumina and Magnesium Trisilicate Oral Suspension

» Alumina and Magnesium Trisilicate Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide [ $\text{Al}(\text{OH})_3$ ] and magnesium trisilicate ( $\text{Mg}_2\text{Si}_2\text{O}_7$ ).

**Packaging and storage**—Preserve in tight containers.

#### Identification—

**A:** To a mixture of 5 mL in 10 mL of 3 N hydrochloric acid add 5 drops of methyl red TS, heat to boiling, add 6 N ammonium hydroxide until the color of the solution changes to deep yellow, then continue boiling for 2 minutes, and filter: the filtrate responds to the tests for *Magnesium* (191).

**B:** Wash the solids on the filter obtained in *Identification* test A with hot ammonium chloride solution (1 in 50), add 10 mL of 3 N hydrochloric acid, and filter: the filtrate responds to the tests for *Aluminum* (191).

**C:** Transfer the filter paper and contents from *Identification* test B to a small platinum dish, ignite, cool in a desiccator, and weigh. Moisten the residue with water and add 6 mL of hydrofluoric acid. Evaporate to dryness, ignite for 5 minutes, cool in a desiccator, and weigh: a loss of more



than 10% in relation to the weight of the residue from the initial ignition indicates  $\text{SiO}_2$ .

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

**pH** (791): between 7.5 and 8.5.

**Assay for aluminum hydroxide—**

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

*Assay preparation*—Transfer about 10 g of well-shaken Oral Suspension to a tared beaker, and weigh accurately. Add 50 mL of water and 10 mL of hydrochloric acid, and digest on a steam bath for 1 hour. Cool, and filter into a 200-mL volumetric flask, washing the filter with water into the flask. Dilute with water to volume, and mix.

*Procedure*—Pipet 20 mL of *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid-ammonium acetate buffer TS, and heat near the boiling point for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 20 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

**Assay for magnesium trisilicate—**

*Assay preparation*—Prepare as directed in the Assay for aluminum hydroxide.

*Procedure*—Pipet 20 mL of *Assay preparation* into a 400-mL beaker, add 180 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia-ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 20 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 6.521 mg of  $\text{Mg}_2\text{Si}_3\text{O}_8$ .

## Alumina and Magnesium Trisilicate Tablets

» Alumina and Magnesium Trisilicate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$  and magnesium trisilicate ( $\text{Mg}_2\text{Si}_3\text{O}_8$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Tablets prepared with the use of *Dried Aluminum Hydroxide Gel* may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ . Tablets intended for the temporary relief of heartburn (acid indigestion) due to acid reflux are so labeled. Tablets that must be chewed before swallowing are so labeled.

**Identification**—One powdered Tablet responds to the Identification tests under *Alumina and Magnesium Trisilicate Oral Suspension*.

**Disintegration** (701): 10 minutes, simulated gastric fluid TS being substituted for water in the test. [NOTE—Tablets that must be chewed before swallowing are exempt from this requirement.]

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium trisilicate.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling. [NOTE—Tablets labeled for the temporary relief of heartburn (acid indigestion) due to acid reflux are exempt from this requirement.]

**Foam** [where Tablets are labeled for the temporary relief of heartburn (acid indigestion) due to acid reflux]—Finely powder a number of Tablets, accurately counted, equivalent to the minimum single dose recommended in the labeling, and transfer the powder to a 100-mL beaker having an inside diameter of 45 mm. Add 5 mL of alcohol and sufficient water to make 40 mL. Mix at 300 rpm for 60 seconds, using a magnetic stirrer and a 9.5- × 38-mm polytetrafluoroethylene-coated stirring bar. Stop the stirrer, and carefully add 10 mL of 0.5 N hydrochloric acid down the side of the beaker. Stir for 30 seconds at 300 rpm. Allow to stand for 10 minutes, and measure the thickness of the foam layer above the liquid in the beaker: the thickness of the foam is not less than 10 mm.

**pH** (791) [where Tablets are labeled for the temporary relief of heartburn (acid indigestion) due to acid reflux]: not less than 4.5, determined on the foam layer obtained in the *Foam* test. [NOTE—Take care that the electrodes do not touch the liquid beneath the foam.]

**Assay for aluminum hydroxide—**

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600 mg of aluminum hydroxide, to a beaker, add 20 mL of water, stir, and slowly add 40 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the flask, add water to volume, and mix.

*Procedure*—Pipet 10 mL of *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of 0.05 M *Edetate disodium titrant* and 20 mL of acetic acid-ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

**Assay for magnesium trisilicate—**

*Potassium chloride solution*—Prepare a solution in water containing 5 g of potassium chloride per 100 mL.

*Magnesium standard solution*—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 50 mL of water, and slowly add 10 mL of hydrochloric acid. Dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 500-mL volumetric flask, dilute with water to volume, and mix.

*Standard preparations*—Transfer 16.0 mL, 18.0 mL, and 20.0 mL of *Magnesium standard solution* to separate 100-mL volumetric flasks, add 2.0 mL of *Potassium chloride solution* to each flask, dilute with water to volume, and mix. These *Standard preparations* contain 1.6, 1.8, and 2.0 µg of magnesium per mL, respectively. [NOTE—Prepare these solutions on the day of use.]

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of



the powder, equivalent to about 5 mg of magnesium trisilicate, to a 100-mL volumetric flask, and add 10 mL of 18 N sulfuric acid. Heat on a steam bath for 30 minutes with occasional swirling. Allow to cool, dilute with water to volume, and mix. Filter this solution, discarding the first 20 mL of the filtrate. Transfer 20.0 mL of the filtrate to a second 100-mL volumetric flask, add 2.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbance of the *Standard preparations* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with an atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)), equipped with a magnesium hollow-cathode lamp and a nitrous oxide–acetylene flame, using water as the blank. Plot the absorbances of the *Standard preparations*, in  $\mu\text{g}$  per mL, of magnesium, and draw the line best fitting the three plotted points. From the graph so obtained determine the concentration,  $C$ , in  $\mu\text{g}$  per mL, of magnesium in the *Assay preparation*. Calculate the quantity, in mg, of magnesium trisilicate ( $\text{Mg}_2\text{Si}_3\text{O}_8$ ) in the portion of Tablets taken by the formula:

$$0.5C(260.86 / 48.62)$$

in which 260.86 is the molecular weight of anhydrous magnesium trisilicate and 48.62 is twice the atomic weight of magnesium.

## Aluminum Acetate Topical Solution

### DEFINITION

Aluminum Acetate Topical Solution yields NLT 1.20 g and NMT 1.45 g of aluminum oxide ( $\text{Al}_2\text{O}_3$ ) and NLT 4.24 g and NMT 5.12 g of acetic acid ( $\text{C}_2\text{H}_4\text{O}_2$ ), corresponding to NLT 4.8 g and NMT 5.8 g of aluminum acetate ( $\text{C}_6\text{H}_9\text{AlO}_6$ ) in each 100 mL. Aluminum Acetate Topical Solution may be stabilized by the addition of NMT 0.6% of boric acid ( $\text{H}_3\text{BO}_3$ ).

Aluminum Subacetate Topical Solution	545 mL
Glacial Acetic Acid	15 mL
Purified Water, a sufficient quantity to make	1000 mL

Add the *Glacial Acetic Acid* to the *Aluminum Subacetate Topical Solution* and sufficient *Purified Water* to bring to final volume. Mix, and filter if necessary. Dispense only clear Aluminum Acetate Topical Solution.

### IDENTIFICATION

- A. IDENTIFICATION TESTS—GENERAL**, *Acetate* (191) and *Aluminum* (191): It meets the requirements of the test for *Aluminum* and for the ferric chloride test for *Acetate*. Ferric chloride TS produces a deep red color that is destroyed by the addition of a mineral acid.

### ASSAY

#### • ALUMINUM OXIDE

**Edetate disodium titrant:** Prepare and standardize 0.05 M edetate disodium titrant as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.

**Sample:** 25 mL

**Blank:** 25 mL of water

**Titrimetric system**

**Mode:** Residual titration

**Back-titrant:** 0.05 M zinc sulfate VS

**Endpoint detection:** Visual

**Analysis:** Pipet the *Sample* into a 250-mL volumetric flask, add 5 mL of hydrochloric acid, and dilute with water to volume. Pipet 25 mL of this solution into a 250-mL beaker, and add, in the order named and with

continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, then heat the solution near the boiling point for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the solution with *Back-titrant* to a bright rose-pink color. Perform a blank determination, and make any necessary correction. Each mL of *Edetate disodium titrant* is equivalent to 2.549 mg of aluminum oxide ( $\text{Al}_2\text{O}_3$ ).

**Acceptance criteria:** 1.20–1.45 g of aluminum oxide ( $\text{Al}_2\text{O}_3$ ) in 100 mL

#### • ACETIC ACID

**Sample:** 20 mL

**Titrimetric system**

**Mode:** Residual titration

**Titrant:** 0.5 N sodium hydroxide VS

**Back-titrant:** 0.5 N sulfuric acid VS

**Endpoint detection:** Visual

**Analysis:** Pipet the *Sample* into a Kjeldahl flask containing a mixture of 20 mL of phosphoric acid and 150 mL of water. Connect the flask to a condenser, the delivery tube from which dips beneath the surface of 50.0 mL of *Titrant* contained in a receiving flask. Distill about 160 mL, then remove the delivery tube from below the surface of the liquid. Allow the distilling flask to cool, add 50 mL of water, and distill an additional 40–45 mL into the receiving flask. Add phenolphthalein TS to the distillate, and titrate the excess *Titrant* with *Back-titrant*. Each mL of *Titrant* is equivalent to 30.03 mg of acetic acid ( $\text{C}_2\text{H}_4\text{O}_2$ ).

**Acceptance criteria:** 4.24–5.12 g of acetic acid ( $\text{C}_2\text{H}_4\text{O}_2$ ) in 100 mL

### OTHER COMPONENTS

#### • LIMIT OF BORIC ACID

**Sample:** 25 mL

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.5 N sodium hydroxide VS

**Endpoint detection:** Visual

**Analysis:** Pipet the *Sample* into 75 mL of water in a conical flask. Add 3 mL of phenolphthalein TS, and add *Titrant* from a buret until a faint pink color is obtained. Heat to boiling, and again neutralize. Add 150 mL of glycerin to the neutralized solution, and titrate with *Titrant*. Perform a blank determination in a similar manner. Subtract the volume of *Titrant* used in the blank from the volume of *Titrant* used after the addition of the glycerin. Each mL of *Titrant* is equivalent to 30.92 mg of boric acid ( $\text{H}_3\text{BO}_3$ ).

**Acceptance criteria:** NMT 0.6% of boric acid ( $\text{H}_3\text{BO}_3$ )

### IMPURITIES

**Delete the following:**

#### • HEAVY METALS (231)

**Test preparation:** 2 mL diluted with water to 25 mL

**Acceptance criteria:** NMT 10 ppm • (Official 1-Jan-2018)

### SPECIFIC TESTS

- PH (791):** 3.6–4.4

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Package in tight containers.

## Aluminum Chloride

$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  241.43

$\text{AlCl}_3$  133.34

Aluminum chloride, hexahydrate;



Aluminum chloride hexahydrate [7784-13-6].  
Anhydrous [7446-70-0].

### DEFINITION

Aluminum Chloride contains NLT 95.0% and NMT 102.0% of aluminum chloride ( $\text{AlCl}_3$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Aluminum (191) and Chloride (191)**  
Sample solution: 100 mg/mL  
Acceptance criteria: Meets the requirements

### ASSAY

#### • PROCEDURE

**Edate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*.

**Sample solution:** 20 mg/mL of aluminum chloride in water

**Titrimetric system**

**Mode:** Back-titration

**Titrant:** 0.05 M zinc sulfate VS

**Endpoint detection:** Visual

**Analysis:** Transfer 10.0 mL of the *Sample solution* into a 250-mL beaker, and add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and boil gently for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the excess edetate disodium with *Titrant* to a bright rose-pink color. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of *Edetate disodium titrant* consumed is equivalent to 6.667 mg of aluminum chloride ( $\text{AlCl}_3$ ).  
**Acceptance criteria:** 95.0%–102.0% on the anhydrous basis

### IMPURITIES

#### • LIMIT OF SULFATE

**Sample solution:** 10 mg/mL

**Analysis:** Add 0.2 mL of barium chloride TS to 10 mL of the *Sample solution*.

**Acceptance criteria:** No turbidity is produced within 1 min.

#### • IRON (241)

**Sample:** 1.0 g

**Analysis:** Dissolve the *Sample* in 45 mL of water, and add 2 mL of hydrochloric acid.

**Acceptance criteria:** NMT 10 ppm

### Delete the following:

#### • HEAVY METALS, Method I (231)

**Test preparation:** Dissolve 1 g of sample in 1 mL of 1 N acetic acid, and sufficient water to make 25 mL.

**Acceptance criteria:** NMT 20 ppm (Official 1-Jan-2018)

#### • LIMIT OF ALKALIES AND ALKALINE EARTHS

**Sample:** 1.0 g

**Analysis:** To a boiling solution of the *Sample* in 150 mL of water add a few drops of methyl red TS, then add 6 N ammonium hydroxide until the color of the solution just changes to a distinct yellow. Add hot water to restore the volume to 150 mL, and filter while hot. Evaporate 75 mL of the filtrate to dryness, and ignite to a constant weight.

**Acceptance criteria:** 0.5%; the weight of the residue is NMT 2.5 mg.

### SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** 42.0%–48.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

## Aluminum Chlorohydrate

$\text{Al}_x(\text{OH})_{3y-z}\text{Cl}_z \cdot \text{H}_2\text{O}$

$\text{Al}_x(\text{OH})_{3y-z}\text{Cl}_z \cdot 2\text{H}_2\text{O}$

210.48

$\text{Al}_x(\text{OH})_{3y-z}\text{Cl}_z$

174.45

Aluminum chlorohydroxide, dihydrate;  
Aluminum hydroxychloride, dihydrate;  
Aluminum chlorohydroxide;  
Aluminum hydroxychloride;  
Dihydrate [12359-72-7].  
Anhydrous [12042-91-0].

### DEFINITION

Aluminum Chlorohydrate consists of complex basic aluminum chloride that is polymeric and loosely hydrated and encompasses a range of aluminum-to-chloride atomic ratios between 1.91:1 and 2.10:1. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous aluminum chlorohydrate [ $\text{Al}_x(\text{OH})_{3y-z}\text{Cl}_z$ ].

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Aluminum (191) and Chloride (191)**  
Sample solution: 100 mg/mL  
Acceptance criteria: Meets the requirements

### ASSAY

#### • PROCEDURE 1: CONTENT OF CHLORIDE

**Sample:** 700 mg

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N silver nitrate VS

**Electrode system:** A glass silver–silver chloride electrode and a silver billet electrode system

**Endpoint detection:** Potentiometric

**Analysis:** Transfer the *Sample* to a 250-mL beaker and add 100 mL of water and 10 mL of diluted nitric acid with stirring. Titrate with *Titrant* and determine the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Use the chloride content thus obtained to calculate the aluminum:chloride atomic ratio.

#### • PROCEDURE 2: CONTENT OF ALUMINUM

**Edetate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*, except use 37.2 g of edetate disodium.

**Sample solution:** Transfer 200 mg of Aluminum Chlorohydrate to a 250-mL beaker, add 20 mL of water and 5 mL of hydrochloric acid, boil on a hot plate for NLT 5 min, and allow to cool.

**Titrimetric system**

**Mode:** Back-titration

**Titrant:** 0.1 M zinc sulfate VS

**Endpoint detection:** Visual

**Analysis:** To the *Sample solution* add 25.0 mL of *Edetate disodium titrant*, and adjust with 2.5 N ammonium hydroxide or 1 N acetic acid to a pH of  $4.7 \pm 0.1$ . Add 20 mL of acetic acid–ammonium acetate buffer TS, 50 mL of alcohol, and 5 mL of dithizone TS. The pH of this solution should be  $4.7 \pm 0.1$ . Titrate the excess edetate disodium with *Titrant* until the color changes from green-violet to rose-pink. Perform a blank titration, and make any necessary correction. Each mL of 0.1 M *Ede-*



tate disodium titrant consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the aluminum:chloride atomic ratio.

• **PROCEDURE 3: ALUMINUM:CHLORIDE ATOMIC RATIO**

**Analysis:** Use the percentage of aluminum found in the test for *Content of Aluminum* and the percentage of chloride found in the test for *Content of Chloride*.

Calculate the aluminum:chloride atomic ratio ( $X$ ) as follows:

$$\text{Result} = (p_{\text{Al}}/p_{\text{Cl}}) \times (A_{\text{Cl}}/A_{\text{Al}})$$

$p_{\text{Al}}$  = percentage of aluminum found in *Content of Aluminum*

$p_{\text{Cl}}$  = percentage of chloride found in *Content of Chloride*

$A_{\text{Cl}}$  = atomic weight of chlorine (Cl), 35.453

$A_{\text{Al}}$  = atomic weight of aluminum (Al), 26.98

**Acceptance criteria:** Between 1.91:1 and 2.10:1

• **PROCEDURE 4**

**Analysis:** Calculate the percentage of anhydrous aluminum chlorohydrate [ $\text{Al}_x(\text{OH})_{3y-z}\text{Cl}_z$ ] in the portion of Aluminum Chlorohydrate taken:

$$\text{Result} = P_{\text{Al}}\{[A_{\text{Al}}X + [M(3X - 1)] + A_{\text{Cl}}]/A_{\text{Al}}X\}$$

$P_{\text{Al}}$  = percentage of aluminum as obtained in the test for *Content of Aluminum*

$A_{\text{Al}}$  = atomic weight of aluminum (Al), 26.98

$X$  = aluminum:chloride atomic ratio, as determined in the test for *Aluminum:Chloride Atomic Ratio*

$M$  = molecular weight of the hydroxide anion (OH), 17.01

$A_{\text{Cl}}$  = atomic weight of chlorine (Cl), 35.453

**Acceptance criteria:** 90.0%–110.0% on the anhydrous basis

**IMPURITIES**

- **ARSENIC**, *Method I* (211): NMT 2 ppm

**Delete the following:**

- **HEAVY METALS**, *Method I* (231): NMT 20 ppm (Official 1-Jan-2018)

- **LIMIT OF IRON**

**Standard solution:** Transfer 2.0 mL of *Standard Iron Solution*, prepared as directed in *Iron* (241), to a 50-mL beaker.

**Sample solution:** Transfer 2.7 g of Aluminum Chlorohydrate to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL beaker.

**Analysis:** To each of the beakers containing the *Standard solution* and the *Sample solution*, add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3–5 min. Allow to cool. Add 5 mL of *Ammonium Thiocyanate Solution* (prepared as directed in *Iron* (241)), transfer to separate 50-mL color comparison tubes, and dilute with water to volume.

**Acceptance criteria:** 150 ppm; the color of the solution from the *Sample solution* is not darker than that of the solution from the *Standard solution*.

**SPECIFIC TESTS**

- **PH** (791)

**Sample solution:** 15 g of Aluminum Chlorohydrate in 100 g of water

**Acceptance criteria:** 3.0–5.0

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** The label states the content of anhydrous aluminum chlorohydrate.

## Aluminum Chlorohydrate Solution

**DEFINITION**

Aluminum Chlorohydrate Solution consists of complex basic aluminum chloride that is polymeric and encompasses a range of aluminum-to-chloride ratios between 1.91:1 and 2.10:1. The following solvents may be used: water, propylene glycol, dipropylene glycol, or alcohol. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled concentration of anhydrous aluminum chlorohydrate ( $\text{Al}_x(\text{OH})_{3y-z}\text{Cl}_z$ ).

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL**, *Aluminum* (191) and *Chloride* (191)

**Sample solution:** Nominally equivalent to 100 mg/mL of anhydrous aluminum chlorohydrate

**Acceptance criteria:** Meets the requirements

- **B. IDENTIFICATION OF PROPYLENE GLYCOL**

Perform this test where propylene glycol is stated on the label.

**Sample solution:** 2 g of Solution in 10 mL of isopropyl alcohol. Mix, filter, and evaporate the filtrate to 1 mL on a steam bath.

**Acceptance criteria:** The IR spectrum of a film of the *Sample solution* on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

- **C. IDENTIFICATION OF DIPROPYLENE GLYCOL**

Perform this test where dipropylene glycol is stated on the label.

**Sample solution:** 2 g of Solution in 10 mL of isopropyl alcohol. Mix, filter, and evaporate the filtrate to 1 mL on a steam bath.

**Acceptance criteria:** The IR spectrum of a film of the *Sample solution* on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

- **D. IDENTIFICATION OF ALCOHOL**

Perform this test where alcohol is stated on the label.

**Analysis:** Mix 5 drops of Solution in a small beaker with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid, and cover the beaker immediately with filter paper moistened with a freshly prepared solution of 0.1 g of sodium nitroferrocyanide and 0.25 g of piperazine in 5 mL of water.

**Acceptance criteria:** An intense blue color is produced on the filter paper, the color becoming paler after a few min.

**ASSAY**

- **PROCEDURE 1: CONTENT OF CHLORIDE**

**Sample:** 1.4 g of Solution

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N silver nitrate VS

**Electrode system:** A silver–silver chloride glass electrode and a silver billet electrode system

**Endpoint detection:** Potentiometric

**Analysis:** Transfer the *Sample* to a 250-mL beaker, and add 100 mL of water and 10 mL of diluted nitric acid with stirring. Titrate with *Titrant*, and determine the endpoint.

**Acceptance criteria:** Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Use the chloride content thus obtained to calculate the aluminum/chloride atomic ratio.

- **PROCEDURE 2: CONTENT OF ALUMINUM**

**Edetate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*, except use 37.2 g of edetate disodium.



**Sample solution:** Transfer 400 mg of Solution to a 250-mL beaker, add 20 mL of water and 5 mL of hydrochloric acid, boil on a hot plate for NLT 5 min, and allow to cool.

#### Titrimetric system

**Mode:** Back titration

**Titrant:** 0.1 M zinc sulfate VS

**Endpoint detection:** Visual

**Analysis:** To the *Sample solution* add 25.0 mL of *Edetate disodium titrant*, and adjust with 2.5 N ammonium hydroxide or 1 N acetic acid to a pH of  $4.7 \pm 0.1$ . Add 20 mL of acetic acid–ammonium acetate buffer TS, 50 mL of alcohol, and 5 mL of dithizone TS. The pH of this solution should be  $4.7 \pm 0.1$ . Titrate excess edetate disodium with *Titrant* until the color changes from green-violet to rose-pink. Perform a blank titration, and make any necessary correction.

**Acceptance criteria:** Each mL of 0.1 M *Edetate disodium titrant* consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the aluminum/chloride atomic ratio.

#### • PROCEDURE 3: ALUMINUM/CHLORIDE ATOMIC RATIO

**Analysis:** Use the percentage of aluminum found in *Content of Aluminum* and the percentage of chloride found in *Content of Chloride*.

Calculate the aluminum/chloride atomic ratio (X) as follows:

$$\text{Result} = (P_{\text{Al}}/P_{\text{Cl}}) \times (A_{\text{Cl}}/A_{\text{Al}})$$

$P_{\text{Al}}$  = percentage of aluminum found in *Content of Aluminum*

$P_{\text{Cl}}$  = percentage of chloride found in *Content of Chloride*

$A_{\text{Cl}}$  = atomic weight of chlorine (Cl), 35.453

$A_{\text{Al}}$  = atomic weight of aluminum (Al), 26.98

**Acceptance criteria:** 1.91:1 to 2.10:1

#### • PROCEDURE 4

**Analysis:** Calculate the percentage of the labeled concentration of anhydrous aluminum chlorohydrate ( $\text{Al}_x(\text{OH})_{3y-2}\text{Cl}_z$ ) in the portion of Solution taken:

$$\text{Result} = P_{\text{Al}} \times \{[A_{\text{Al}}X + (M(3X - 1)) + A_{\text{Cl}}]/A_{\text{Al}}X\}$$

$P_{\text{Al}}$  = percentage of aluminum found in *Content of Aluminum*

$A_{\text{Al}}$  = atomic weight of aluminum (Al), 26.98

X = aluminum/chloride atomic ratio, as determined in *Aluminum/Chloride Atomic Ratio*

M = molecular weight of the hydroxide anion (OH), 17.01

$A_{\text{Cl}}$  = atomic weight of chlorine (Cl), 35.453

**Acceptance criteria:** 90.0%–110.0%

#### IMPURITIES

• **ARSENIC, Method I (211):** NMT 2 ppm

#### Delete the following:

• **HEAVY METALS, Method I (231):** NMT 10 ppm • (Official 1-Jan-2018)

#### • LIMIT OF IRON

**Standard preparation:** 2.0 mL of *Standard Iron Solution*, prepared as directed in *Iron (241)*

**Test preparation:** Transfer 5.3 g of Solution to a 100-mL volumetric flask, and dilute with water to volume.

**Analysis:** Transfer 2.0 mL of the *Standard preparation* into a 50-mL beaker. Transfer 5.0 mL of the *Test preparation* into a second 50-mL beaker. To each of the beakers add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3–5 min. Allow to cool. Add 5 mL of *Ammonium Thiocyanate Solution*, prepared as directed in *Iron (241)*, transfer to separate

50-mL color-comparison tubes, and dilute with water to volume.

**Acceptance criteria:** 75 ppm; the color of the solution from the *Test preparation* is not darker than that from the *Standard preparation*.

#### SPECIFIC TESTS

##### • PH (791)

**Sample solution:** Dilute 3 g of Solution with water to 10 mL.

**Acceptance criteria:** 3.0–5.0

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum chlorohydrate contained therein.

### Aluminum Chlorohydrate Polyethylene Glycol



Aluminum chlorohydroxide polyethylene glycol complex.

Aluminum hydroxychloride polyethylene glycol complex.

» Aluminum Chlorohydrate Polyethylene Glycol consists of aluminum chlorohydrate in which some of the waters of hydration have been replaced by polyethylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 1.91:1 and 2.10:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum chlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum chlorohydrate.

#### Identification—

A: A solution (1 in 10) responds to the tests for *Aluminum (191)* and for *Chloride (191)*.

B: *Infrared Absorption (197F)*—

**Test specimen**—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

**Standard specimen:** a similar preparation of polyethylene glycol.

**pH (791):** between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I (211):** 2 µg per g.

#### Delete the following:

• **Heavy metals, Method I (231):** 20 µg per g • (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Chlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Chlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum*



under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Chlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.91:1 and 2.10:1.

**Assay**—Calculate the percentage of anhydrous aluminum chlorohydrate in the Aluminum Chlorohydrate Polyethylene Glycol by the formula:

$$Al\{[26.98x + [17.01(3x - 1)] + 35.453] / 26.98x\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Chlorohydrate Propylene Glycol

$Al_2(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mC_3H_8O_2$

$Al_2(H_2O)_{y-z}(OH)_{6-n}(Cl)_n(C_3H_8O_2)_m$

Aluminum chlorohydroxide, hydrate: propylene glycol complex (1:1).

Aluminum hydroxychloride, hydrate: propylene glycol complex (1:1) [53026-85-0].

» Aluminum Chlorohydrate Propylene Glycol is a complex of aluminum chlorohydrate and propylene glycol in which some of the waters of hydration of the aluminum chlorohydrate have been replaced by propylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum chlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum chlorohydrate.

### Identification—

**A:** A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**B:** *Infrared Absorption* (197F)—

**Test specimen**—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

**Standard specimen**: a similar preparation of propylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231): 20 µg per g. • (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Chlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

### Content of aluminum—

**Edetate disodium titrant**—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*, except to use 37.2 g of edetate disodium instead of 18.6 g.

**Test solution**—Transfer about 1.6 g of Aluminum Chlorohydrate Propylene Glycol, accurately weighed, to a 100-mL beaker, add 15 to 20 mL of water and 5 to 6 mL of hydrochloric acid, and boil on a hot plate for 15 to 20 minutes. Cool the solution, and with the aid of water transfer to a 100-mL volumetric flask. Dilute with water to volume, and mix.

**Procedure**—Transfer 5.0 mL of the *Test solution* to a 250-mL beaker, add 10 to 15 mL of water, and adjust with 1 N sodium hydroxide to a pH of  $1.5 \pm 0.5$ . Add 10.0 mL of *Edetate disodium titrant*, and heat to boiling. Cool the solution and carefully introduce a magnetic stirring bar into the beaker. Add 10 to 15 mL of acetic acid–ammonium acetate buffer TS, 40 to 50 mL of alcohol, and while stirring adjust with glacial acetic acid to a pH of  $4.6 \pm 0.1$ . Add 1 to 2 mL of dithizone TS and 40 to 50 mL of alcohol, and titrate with 0.1 M zinc sulfate VS until the color changes from a green-violet to a rose-pink. Perform a blank titration, and make any necessary correction. Each mL of 0.1 M *Edetate disodium titrant* consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Chlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the chloride content thus obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the *Assay* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.91:1 and 2.1:1.

**Assay**—Calculate the percentage of anhydrous aluminum chlorohydrate in the Aluminum Chlorohydrate Propylene Glycol by the formula:

$$Al\{[26.98x + [17.01(3x - 1)] + 35.453] / 26.98x\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide ion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Dichlorohydrate

$Al_2(OH)_{3y-z}Cl_z \cdot nH_2O$

Aluminum chlorohydroxide;

Aluminum hydroxychloride.

### DEFINITION

Aluminum Dichlorohydrate consists of complex basic aluminum chloride that is polymeric and loosely hydrated and encompasses a range of aluminum-to-chloride atomic ra-



tios between 0.90:1 and 1.25:1. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous aluminum dichlorohydrate  $[\text{Al}_2(\text{OH})_3\text{Cl}_2]$ .

## IDENTIFICATION

### • A. IDENTIFICATION TESTS—GENERAL, Aluminum (191) and Chloride (191)

Sample solution: 100 mg/mL

Acceptance criteria: Meets the requirements

## ASSAY

### • PROCEDURE 1: CONTENT OF CHLORIDE

Sample: 700 mg

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N silver nitrate VS

Electrode system: A glass silver-silver chloride electrode and a silver billet electrode system

Endpoint detection: Potentiometric

Analysis: Transfer the Sample to a 250-mL beaker, and add 100 mL of water and 10 mL of diluted nitric acid with stirring. Titrate with Titrant, and determine the endpoint potentiometrically. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Use the chloride content thus obtained to calculate the aluminum:chloride atomic ratio.

### • PROCEDURE 2: CONTENT OF ALUMINUM

EDETATE DISODIUM TITRANT: Prepare and standardize as directed in *Reagents, Volumetric Solutions, Edetate Disodium, Twentieth-Molar (0.05 M)*, except use 37.2 g of edetate disodium.

Sample solution: Transfer 200 mg of Aluminum Dichlorohydrate to a 250-mL beaker, add 20 mL of water and 5 mL of hydrochloric acid, boil on a hot plate for NLT 5 min, and allow to cool.

Titrimetric system

Mode: Back-titration

Titrant: 0.1 M zinc sulfate VS

Endpoint detection: Visual

Analysis: To the Sample solution add 25.0 mL of Edetate disodium titrant, and adjust with 2.5 N ammonium hydroxide or 1 N acetic acid to a pH of  $4.7 \pm 0.1$ . Add 20 mL of acetic acid-ammonium acetate buffer TS, 50 mL of alcohol, and 5 mL of dithizone TS. The pH of this solution should be  $4.7 \pm 0.1$ . Titrate the excess edetate disodium with Titrant until the color changes from green-violet to rose-pink. Perform a blank titration, and make any necessary correction. Each mL of 0.1 M Edetate disodium titrant consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the aluminum:chloride atomic ratio.

### • PROCEDURE 3: ALUMINUM:CHLORIDE ATOMIC RATIO

Analysis: Use the percentage of aluminum found in the test for *Content of Aluminum* and the percentage of chloride found in the test for *Content of Chloride*. Calculate the aluminum:chloride atomic ratio (X) as follows:

$$\text{Result} = (p_{\text{Al}}/p_{\text{Cl}}) \times (A_{\text{Cl}}/A_{\text{Al}})$$

$p_{\text{Al}}$  = percentage of aluminum found in *Content of Aluminum*

$p_{\text{Cl}}$  = percentage of chloride found in *Content of Chloride*

$A_{\text{Cl}}$  = atomic weight of chlorine (Cl), 35.453

$A_{\text{Al}}$  = atomic weight of aluminum (Al), 26.98

Acceptance criteria: Between 0.90:1 and 1.25:1

### • PROCEDURE 4

Analysis: Calculate the percentage of anhydrous aluminum dichlorohydrate  $[\text{Al}_2(\text{OH})_3\text{Cl}_2]$  in the portion of Aluminum Dichlorohydrate taken:

$$\text{Result} = P_{\text{Al}}\{A_{\text{Al}}X + [M(3X - 1)] + A_{\text{Cl}}\}/A_{\text{Al}}X$$

$P_{\text{Al}}$  = percentage of aluminum as obtained in the test for *Content of Aluminum*

$A_{\text{Al}}$  = atomic weight of aluminum (Al), 26.98

X = aluminum:chloride atomic ratio, as determined in the test for *Aluminum:Chloride Atomic Ratio*

M = molecular weight of the hydroxide anion (OH), 17.01

$A_{\text{Cl}}$  = atomic weight of chlorine (Cl), 35.453

Acceptance criteria: 90.0%–110.0% on the anhydrous basis

## IMPURITIES

### • ARSENIC, Method I (211): NMT 2 ppm

### Delete the following:

### • HEAVY METALS, Method I (231): NMT 20 ppm • (Official 1: Jan-2018)

### • LIMIT OF IRON

Standard solution: Transfer 2.0 mL of *Standard Iron Solution*, prepared as directed in *Iron (241)*, to a 50-mL beaker.

Sample solution: Transfer 2.7 g of Aluminum Dichlorohydrate to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL beaker.

Analysis: To each of the beakers containing the Standard solution and the Sample solution, add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3–5 min. Allow to cool. Add 5 mL of *Ammonium Thiocyanate Solution* (prepared as directed in *Iron (241)*), transfer to separate 50-mL color comparison tubes, and dilute with water to volume.

Acceptance criteria: 150 ppm; the color of the solution from the Sample solution is not darker than that of the solution from the Standard solution.

## SPECIFIC TESTS

### • pH (791)

Sample solution: 15 g of Aluminum Dichlorohydrate in 100 g of water

Acceptance criteria: 3.0–5.0

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in well-closed containers.

### • LABELING: The label states the content of anhydrous aluminum dichlorohydrate.

## Aluminum Dichlorohydrate Solution

### DEFINITION

Aluminum Dichlorohydrate Solution consists of complex basic aluminum chloride that is polymeric and encompasses a range of aluminum-to-chloride atomic ratios between 0.90:1 and 1.25:1. The following solvents may be used: water, propylene glycol, dipropylene glycol, or alcohol. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled concentration of anhydrous aluminum dichlorohydrate  $[\text{Al}_2(\text{OH})_3\text{Cl}_2]$ .

## IDENTIFICATION

### • A. IDENTIFICATION TESTS—GENERAL, Aluminum (191) and Chloride (191)

Sample solution: Nominally equivalent to 100 mg/mL of anhydrous aluminum dichlorohydrate

Acceptance criteria: Meets the requirements

### • B. INFRARED ABSORPTION (197F): (where propylene glycol is indicated on the label)

Sample solution: Add 10 mL of isopropyl alcohol to 2 g of Solution, and filter. Evaporate the filtrate to 1 mL on



a steam bath. Deposit this solution on a silver chloride disk.

**Standard solution:** A similar preparation of propylene glycol

**Acceptance criteria:** Meets the requirements

- **C. INFRARED ABSORPTION** (197F): (where dipropylene glycol is indicated on the label)

**Sample solution:** Add 10 mL of isopropyl alcohol to 2 g of Solution, and filter. Evaporate the filtrate to 1 mL on a steam bath. Deposit this solution on a silver chloride disk.

**Standard solution:** A similar preparation of dipropylene glycol

**Acceptance criteria:** Meets the requirements

- **D. IDENTIFICATION OF ALCOHOL**

Perform this test where alcohol is stated on the label.

**Analysis:** Mix 5 drops of Solution in a small beaker with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid, and cover the beaker immediately with filter paper moistened with a freshly prepared solution of 0.1 g of sodium nitroferricyanide and 0.25 g of piperazine in 5 mL of water.

**Acceptance criteria:** An intense blue color is produced on the filter paper, the color becoming paler after a few min.

## ASSAY

- **PROCEDURE 1: CONTENT OF CHLORIDE**

**Sample:** 1.4 g of Solution

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N silver nitrate VS

**Electrode system:** A silver-silver chloride glass electrode and a silver billet electrode system

**Endpoint detection:** Potentiometric

**Analysis:** Transfer the *Sample* to a 250-mL beaker, and add 100 mL of water and 10 mL of diluted nitric acid with stirring. Titrate with *Titrant*, and determine the endpoint.

**Acceptance criteria:** Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride (Cl). Use the chloride content thus obtained to calculate the aluminum/chloride atomic ratio.

- **PROCEDURE 2: CONTENT OF ALUMINUM**

**Eдетate disodium titrant:** Prepare and standardize as directed in *Reagents, Volumetric Solutions, Eдетate Disodium, Twentieth-Molar (0.05 M)*, except use 37.2 g of edetate disodium.

**Sample solution:** Transfer 400 mg of Solution to a 250-mL beaker, add 20 mL of water and 5 mL of hydrochloric acid, boil on a hot plate for NLT 5 min, and allow to cool.

**Titrimetric system**

**Mode:** Back titration

**Titrant:** 0.1 M zinc sulfate VS

**Endpoint detection:** Visual

**Analysis:** To the *Sample solution* add 25.0 mL of *Eдетate disodium titrant*, and adjust with 2.5 N ammonium hydroxide or 1 N acetic acid to a pH of  $4.7 \pm 0.1$ . Add 20 mL of acetic acid-ammonium acetate buffer TS, 50 mL of alcohol, and 5 mL of dithizone TS. The pH of this solution should be  $4.7 \pm 0.1$ . Titrate excess edetate disodium with *Titrant* until the color changes from green-violet to rose-pink. Perform a blank titration, and make any necessary correction.

**Acceptance criteria:** Each mL of 0.1 M *Eдетate disodium titrant* consumed is equivalent to 2.698 mg of aluminum (Al). Use the aluminum content thus obtained to calculate the aluminum/chloride atomic ratio.

- **PROCEDURE 3: ALUMINUM/CHLORIDE ATOMIC RATIO**

**Analysis:** Use the percentage of aluminum found in *Content of Aluminum* and the percentage of chloride found in *Content of Chloride*.

Calculate the aluminum/chloride atomic ratio (X) as follows:

$$\text{Result} = (P_{Al}/P_{Cl}) \times (A_{Cl}/A_{Al})$$

$P_{Al}$  = percentage of aluminum found in *Content of Aluminum*

$P_{Cl}$  = percentage of chloride found in *Content of Chloride*

$A_{Cl}$  = atomic weight of chlorine (Cl), 35.453

$A_{Al}$  = atomic weight of aluminum (Al), 26.98

**Acceptance criteria:** 0.90: 1 to 1.25: 1

- **PROCEDURE 4**

**Analysis:** Calculate the percentage of the labeled concentration of anhydrous aluminum dichlorohydrate ( $Al_2(OH)_3 \cdot 2Cl_2$ ) in the portion of Solution taken:

$$\text{Result} = P_{Al} \times \{[A_{Al}X + (M(3X - 1)) + A_{Cl}]/A_{Al}X\}$$

$P_{Al}$  = percentage of aluminum found in *Content of Aluminum*

$A_{Al}$  = atomic weight of aluminum (Al), 26.98

$X$  = aluminum/chloride atomic ratio, as determined in *Aluminum/Chloride Atomic Ratio*

$M$  = molecular weight of the hydroxide anion (OH), 17.01

$A_{Cl}$  = atomic weight of chlorine (Cl), 35.453

**Acceptance criteria:** 90.0%–110.0%

## IMPURITIES

- **ARSENIC**, *Method I* (211): NMT 2 ppm

## Delete the following:

- **HEAVY METALS**, *Method I* (231): NMT 10 ppm • (Official 1-Jan-2018)

- **LIMIT OF IRON**

**Standard preparation:** 2.0 mL of *Standard Iron Solution*, prepared as directed in *Iron* (241)

**Test preparation:** Transfer 5.3 g of Solution to a 100-mL volumetric flask, and dilute with water to volume.

**Analysis:** Transfer 2.0 mL of the *Standard preparation* into a 50-mL beaker. Transfer 5.0 mL of the *Test preparation* into a second 50-mL beaker. To each of the beakers add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3–5 min. Allow to cool. Add 5 mL of *Ammonium Thiocyanate Solution*, prepared as directed in *Iron* (241), transfer to separate 50-mL color-comparison tubes, and dilute with water to volume.

**Acceptance criteria:** 75 ppm; the color of the solution from the *Test preparation* is not darker than that from the *Standard preparation*.

## SPECIFIC TESTS

- **pH** (791)

**Sample solution:** Dilute 3 g of Solution with water to 10 mL.

**Acceptance criteria:** 3.0–5.0

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum dichlorohydrate contained therein.



## Aluminum Dichlorohydrate Polyethylene Glycol

$\text{Al}_x(\text{OH})_{3y-z}\text{Cl}_z \cdot n\text{H}_2\text{O} \cdot m\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH}$

Aluminum chlorohydroxide polyethylene glycol complex.

Aluminum hydroxychloride polyethylene glycol complex.

» Aluminum Dichlorohydrate Polyethylene Glycol consists of aluminum dichlorohydrate in which some of the waters of hydration have been replaced by polyethylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 0.90:1 and 1.25:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum dichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum dichlorohydrate.

### Identification—

A: A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

B: *Infrared Absorption* (197F)—

**Test specimen**—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

**Standard specimen**: a similar preparation of polyethylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231): 20 µg per g. (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Dichlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Dichlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Dichlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively; the ratio is between 0.90:1 and 1.25:1.

**Assay**—Calculate the percentage of anhydrous aluminum dichlorohydrate in the Aluminum Dichlorohydrate Polyethylene Glycol by the formula:

$$\text{Al} / \{ (26.98x + [17.01(3x - 1)] + 35.453) / 26.98x \}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum-to-chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Dichlorohydrate Propylene Glycol

$\text{Al}_x(\text{OH})_{3y-z}\text{Cl}_z \cdot n\text{H}_2\text{O} \cdot m\text{C}_3\text{H}_8\text{O}_2$

Aluminum chlorohydroxide propylene glycol complex.

Aluminum hydroxychloride propylene glycol complex.

» Aluminum Dichlorohydrate Propylene Glycol consists of aluminum dichlorohydrate in which some of the waters of hydration have been replaced by propylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 0.90:1 and 1.25:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum dichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum dichlorohydrate.

### Identification—

A: A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

B: Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231): 20 µg per g. (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Dichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Dichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Dichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.



**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively; the ratio is between 0.90:1 and 1.25:1.

**Assay**—Calculate the percentage of anhydrous aluminum dichlorohydrate in the Aluminum Dichlorohydrate Propylene Glycol by the formula:

$$Al[(26.98x + [17.01(3x - 1)] + 35.453) / 26.98x]$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum-to-chloride atomic ratio, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

### Aluminum Hydroxide Gel

Al(OH)<sub>3</sub> 78.00

Aluminum hydroxide.

Aluminum hydroxide [21645-51-2].

» Aluminum Hydroxide Gel is a suspension of amorphous aluminum hydroxide in which there is a partial substitution of carbonate for hydroxide. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide [Al(OH)<sub>3</sub>]. It may contain Peppermint Oil, Glycerin, Sorbitol, Sucrose, Saccharin, or other suitable flavors, and it may contain suitable antimicrobial agents.

**Packaging and storage**—Preserve in tight containers, and avoid freezing.

#### Identification—

**A:** Place about 1 g in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

**B:** The solution remaining in the flask responds to the tests for *Aluminum* (191).

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—Its total aerobic microbial count does not exceed 100 cfu per mL, and it meets the requirements of the test for the absence of *Escherichia coli*.

**Acid-neutralizing capacity (301)**—Not less than 65.0% of the expected mEq value, calculated from the results of the Assay, is obtained. Each mg of Al(OH)<sub>3</sub> has an expected acid-neutralizing capacity value of 0.0385 mEq.

**pH (791):** between 5.5 and 8.0, determined potentiometrically.

**Chloride**—Transfer an accurately measured quantity of the Gel, equivalent to 0.6 g of Al(OH)<sub>3</sub>, to a porcelain dish. Add 0.1 mL of potassium chromate TS and 25 mL of water. Stir, and add 0.10 N silver nitrate until a faint, persistent pink color is obtained: not more than 8.0 mL of 0.10 N silver nitrate is required [4.7%, based on the Al(OH)<sub>3</sub> content].

**Sulfate (221)**—Add 5.0 mL of 3 N hydrochloric acid to an accurately measured quantity of the Gel, equivalent to 0.3 g of Al(OH)<sub>3</sub>, and heat to dissolve the specimen under test. Cool, dilute with water to 250 mL, and filter if necessary: a 20-mL portion of the filtrate shows no more sulfate than

corresponds to 0.20 mL of 0.020 N sulfuric acid [0.8%, based on the Al(OH)<sub>3</sub> content].

**Arsenic, Method 1 (211)**—Prepare a *Standard Preparation* as directed in the test for *Arsenic* (211), except to prepare it to contain 5 µg of arsenic instead of 3 µg. Prepare the *Test Preparation* as follows. Dissolve an accurately measured quantity of the Gel, equivalent to 0.5 g of Al(OH)<sub>3</sub>, in 20 mL of 7 N sulfuric acid. The limit is 0.001%, based on the Al(OH)<sub>3</sub> content.

#### Delete the following:

• **Heavy metals (231)**—Dissolve an accurately measured quantity of the Gel, equivalent to 0.24 g of Al(OH)<sub>3</sub>, in 10 mL of 3 N hydrochloric acid with the aid of heat, filter, if necessary, and dilute with water to 25 mL: the limit is 0.0083%, based on the Al(OH)<sub>3</sub> content. • (Official 1-Jan-2018)

#### Assay—

**Edetate disodium titrant**—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

**Procedure**—Transfer an accurately measured quantity of Gel, equivalent to about 1.5 g of Al(OH)<sub>3</sub>, to a beaker, add 15 mL of hydrochloric acid, and heat gently until solution is complete. Cool, transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. Pipet 20 mL of this solution into a 250-mL beaker, and add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, then heat the solution near the boiling point for 5 minutes. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the solution with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 20 mL of water for the sample, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* consumed is equivalent to 3.900 mg of Al(OH)<sub>3</sub>.

### Dried Aluminum Hydroxide Gel

Al(OH)<sub>3</sub> 78.00

Aluminum hydroxide [21645-51-2].

» Dried Aluminum Hydroxide Gel is an amorphous form of aluminum hydroxide in which there is a partial substitution of carbonate for hydroxide. It contains the equivalent of not less than 76.5 percent of Al(OH)<sub>3</sub>, and it may contain varying quantities of basic aluminum carbonate and bicarbonate.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where the quantity of dried aluminum hydroxide gel equivalent is stated in the labeling of any preparation, this shall be understood to be on the basis that each mg of dried gel is equivalent to 0.765 mg of Al(OH)<sub>3</sub>.

#### USP Reference standards (11)—

USP Dried Aluminum Hydroxide Gel RS

#### Identification—

**A: Infrared Absorption (197K).**

**B:** Dissolve 500 mg in 10 mL of 3 N hydrochloric acid, with gentle warming: the solution responds to the tests for *Aluminum* (191).

**Acid-neutralizing capacity (301):** not less than 25.0 mEq per g, 400 mg being tested as directed for *Powders* under *Test Preparation*.

**pH (791):** not higher than 10.0, in an aqueous dispersion (1 in 25).



**Chloride** (221)—Dissolve 1.0 g in 30 mL of 2 N nitric acid, heat to boiling, add water to make 100 mL, and filter: a 5.0-mL portion of the filtrate, diluted with an equal volume of water, shows no more chloride than corresponds to 0.60 mL of 0.020 N hydrochloric acid (0.85%).

**Sulfate** (221)—Dissolve 330 mg in 15 mL of 3 N hydrochloric acid, heat to boiling, add water to make 250 mL, and filter: a 25-mL portion of the filtrate shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.6%).

**Arsenic, Method I** (211)—Dissolve 1.5 g in 80 mL of 7 N sulfuric acid, and dilute with water to 220 mL: 55 mL of the resulting solution meets the requirements of the test, the addition of 20 mL of 7 N sulfuric acid specified under *Procedure* being omitted. The limit is 8 ppm.

#### Delete the following:

• **Heavy metals** (231)—Dissolve 330 mg in 10 mL of 3 N hydrochloric acid with the aid of heat, filter if necessary, and dilute with water to 25 mL: the limit is 0.006%. • (Official 1-Jan-2018)

#### Assay—

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

*Procedure*—Weigh accurately about 2 g of Gel, and dissolve in 15 mL of hydrochloric acid, with the aid of heat. Cool, transfer to a 500-mL volumetric flask, dilute with water to volume, and mix. Pipet 20 mL of this solution into a 250-mL beaker, and add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, then heat the solution near the boiling point for 5 minutes. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate the solution with 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, substituting 20 mL of water for the sample solution, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

### Dried Aluminum Hydroxide Gel Capsules

» Dried Aluminum Hydroxide Gel Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The Capsules may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ .

#### Identification—

A: Place a portion of Capsule contents, equivalent to about 500 mg of aluminum hydroxide, in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 10 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

B: The solution remaining in the flask responds to the tests for *Aluminum* (191).

**Disintegration** (701): 10 minutes, simulated gastric fluid TS being substituted for water in the test.

**Uniformity of dosage units** (905): meet the requirements.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than 55.0% of the expected mEq value, calculated from the labeled quantity of  $\text{Al}(\text{OH})_3$ , is obtained. Each mg of  $\text{Al}(\text{OH})_3$  has an expected acid-neutralizing capacity value of 0.0385 mEq.

#### Assay—

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

*Procedure*—Weigh accurately the contents of not fewer than 20 Capsules, and mix. Transfer an accurately weighed portion of the powder, equivalent to about 1.2 g of aluminum hydroxide, to a beaker, add 15 mL of hydrochloric acid, and heat until dissolved. Dilute with water to about 100 mL, mix, and filter quantitatively into a 500-mL volumetric flask, washing the filter with water. Proceed as directed in the Assay under *Dried Aluminum Hydroxide Gel*, beginning with "dilute with water to volume." Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

### Dried Aluminum Hydroxide Gel Tablets

» Dried Aluminum Hydroxide Gel Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Tablets may be labeled to state the aluminum hydroxide content in terms of the equivalent amount of dried aluminum hydroxide gel, on the basis that each mg of dried gel is equivalent to 0.765 mg of  $\text{Al}(\text{OH})_3$ .

#### Identification—

A: Place a quantity of finely ground Tablets, equivalent to about 500 mg of aluminum hydroxide, in a flask equipped with a stopper and glass tubing, the tip of which is immersed in calcium hydroxide TS in a test tube. Add 5 mL of 3 N hydrochloric acid to the flask, and immediately insert the stopper: gas evolves in the flask and a precipitate is formed in the test tube.

B: The solution remaining in the flask responds to the tests for *Aluminum* (191).

**Disintegration** (701): 10 minutes, simulated gastric fluid TS being substituted for water in the test.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation*.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and not less than 55.0% of the expected mEq value, calculated from the labeled quantity of  $\text{Al}(\text{OH})_3$ , is obtained. Each mg of  $\text{Al}(\text{OH})_3$  has an expected acid-neutralizing capacity value of 0.0385 mEq.

#### Assay—

*Edetate disodium titrant*—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

*Procedure*—Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 1.2 g of aluminum hydroxide, add 15 mL of hydrochloric acid, and heat until dissolved. Dilute with water to about 100 mL, mix, and filter quantitatively into a 500-mL volumetric flask, washing the filter with water. Proceed as directed in the Assay under *Dried Aluminum Hydroxide Gel*, beginning with "dilute with water to volume." Each



mL of 0.05 M *Edetate disodium* titrant is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

### Aluminum Phosphate Gel

Phosphoric acid, aluminum salt (1:1).  
Aluminum phosphate (1:1) [7784-30-7].

» Aluminum Phosphate Gel is a water suspension containing not less than 4.0 percent and not more than 5.0 percent (w/w) of aluminum phosphate ( $\text{AlPO}_4$ ). It may contain sodium benzoate, benzoic acid, or other suitable agent, in an amount not exceeding 0.5 percent, as a preservative.

**Packaging and storage**—Preserve in tight containers.

#### Identification—

**A:** A solution of it in hydrochloric acid meets the requirements of the tests for *Aluminum* (191).

**B:** A solution of it in 2 N nitric acid meets the requirements of the tests for *Phosphate* (191).

**pH** (791): between 6.0 and 7.2.

**Soluble phosphate**—Filter 20 g, and wash the residue with 30 mL of water. Add to the filtrate 2 mL of nitric acid, heat to 60°, and add 20 mL of ammonium molybdate TS. Heat at 50° for 30 minutes, filter, wash the precipitate with dilute nitric acid (1 in 36), then wash with potassium nitrate solution (1 in 100) until the last portion of the filtrate is not acid to litmus paper. Dissolve the precipitate in 50.0 mL of 0.5 N sodium hydroxide VS, add phenolphthalein TS, and titrate the excess alkali with 0.5 N hydrochloric acid VS. Each mL of 0.5 N sodium hydroxide is equivalent to 2.065 mg of  $\text{PO}_4$ . The soluble phosphate, calculated as  $\text{PO}_4$ , does not exceed 0.30%.

**Sulfate** (221)—Add 10 mL of 3 N hydrochloric acid to 10 g of Gel, and heat to boiling. Cool, dilute with water to 250 mL, and filter, if necessary. A 10-mL portion of the solution shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid: not more than 0.05% is found.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* by dissolving 5.0 g of Gel in the smallest necessary volume of 3 N hydrochloric acid. The limit is 0.6 ppm.

#### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to make the following modifications.

**Standard Preparation**—Into a 50-mL color-comparison tube pipet 4.0 mL of *Standard Lead Solution*, dilute with water to 25 mL, adjust with 6 N ammonium hydroxide to a pH between 1.9 and 2.1, dilute with water to 40 mL, and mix.

**Test Preparation**—Dissolve 8.0 g in 5 mL of 3 N hydrochloric acid, warming if necessary, dilute with water to 25 mL, and adjust with 6 N ammonium hydroxide to a pH between 1.9 and 2.1. Transfer to a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.

**Monitor Preparation**—Into a 50-mL color-comparison tube place 25 mL of the *Test Preparation*, add 4.0 mL of *Standard Lead Solution*, adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 1.9 and 2.1, dilute with water to 40 mL, and mix.

**Procedure**—Proceed as directed in the chapter, except to omit the addition of 2 mL of pH 3.5 *Acetate Buffer*. Not more than 5 µg per g is found. (Official 1-Jan-2018)

**Chloride**—Transfer 25 g to a beaker with the aid of about 50 mL of water, add 5 mL of nitric acid, mix, then add, with stirring, 30.0 mL of 0.1 N silver nitrate VS. Warm on a steam bath for 30 minutes, filter, and wash the precipitate with water acidified with nitric acid. To the filtrate add ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Not more than 0.16% of chloride is found.

**Assay**—To about 20 g of Gel, accurately weighed, in a 100-mL volumetric flask, add nitric acid to effect solution, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 400-mL beaker, dilute with water to 100 mL, heat to 60°, add an excess of ammonium molybdate TS, and maintain at 50° for 30 minutes. Filter, and wash the precipitate with dilute nitric acid (1 in 36), then with potassium nitrate solution (1 in 100) until the last portion of the filtrate is not acid to litmus paper. Dissolve the precipitate in 50.0 mL of 0.5 N sodium hydroxide VS, add phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 N sulfuric acid VS. Each mL of 0.5 N sodium hydroxide is equivalent to 2.651 mg of  $\text{AlPO}_4$ .

### Aluminum Sesquichlorohydrate

$\text{Al}_2(\text{OH})_3\text{Cl}_2 \cdot n\text{H}_2\text{O}$

Aluminum chlorohydroxide.

Aluminum hydroxychloride [11097-68-0].

» Aluminum Sesquichlorohydrate consists of complex basic aluminum chloride that is polymeric and loosely hydrated and encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum sesquichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum sesquichlorohydrate.

**Identification**—A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

#### Delete the following:

• **Heavy metals, Method I** (231): 20 µg per g. (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Sesquichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Sesquichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Sesquichlorohydrate instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum*



by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.26:1 and 1.90:1.

**Assay**—Calculate the percentage of anhydrous aluminum sesquichlorohydrate in the Aluminum Sesquichlorohydrate by the formula:

$$Al\{[26.98x + [17.01(3x - 1)] + 35.453] / 26.98x\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

### Aluminum Sesquichlorohydrate Solution

» Aluminum Sesquichlorohydrate Solution consists of complex basic aluminum chloride that is polymeric and encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. The following solvents may be used: water, propylene glycol, dipropylene glycol, or alcohol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum sesquichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum sesquichlorohydrate contained therein.

#### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum sesquichlorohydrate per mL responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**D:** *Identification of alcohol* (where stated on the label)—Mix 5 drops of Solution in a small beaker with 1 mL of potassium permanganate solution (1 in 100) and 5 drops of 2 N sulfuric acid, and cover the beaker immediately with filter paper moistened with a freshly prepared solution of 0.1 g of sodium nitroferricyanide and 0.25 g of piperazine in 5 mL of water: an intense blue color is produced on the filter paper, the color becoming paler after a few minutes.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

#### Delete the following:

• **Heavy metals, Method I** (231)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 10 µg per g. • (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Sesquichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Limit of iron* under *Aluminum Chlorohydrate Solution*. The limit is 75 µg per g.

**Content of aluminum**—Using Aluminum Sesquichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Content of aluminum* under *Aluminum Chlorohydrate Solution*. Use the result to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Sesquichlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Content of chloride* under *Aluminum Chlorohydrate Solution*. Use the result to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively: the ratio is between 1.26:1 and 1.90:1.

**Assay**—Calculate the percentage of anhydrous aluminum sesquichlorohydrate in the Solution by the formula:

$$Al\{[26.98x + [17.01(3x - 1)] + 35.453] / 26.98x\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

### Aluminum Sesquichlorohydrate Polyethylene Glycol

$Al_y(OH)_{3y-z}Cl_z \cdot nH_2O \cdot mH(OCH_2CH_2)_nOH$   
Aluminum chlorohydroxide polyethylene glycol complex.  
Aluminum hydroxychloride polyethylene glycol complex.

» Aluminum Sesquichlorohydrate Polyethylene Glycol consists of aluminum sesquichlorohydrate in which some of the waters of hydration have been replaced by polyethylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum sesquichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum sesquichlorohydrate.

#### Identification—

**A:** A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).



**B: Infrared Absorption (197F)—**

**Test specimen**—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

**Standard specimen**: a similar preparation of polyethylene glycol.

**pH (791)**: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I (211)**: 2 µg per g.

**Delete the following:**

• **Heavy metals, Method I (231)**: 20 µg per g. (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Sesquichlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Sesquichlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Sesquichlorohydrate Polyethylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively; the ratio is between 1.26:1 and 1.90:1.

**Assay**—Calculate the percentage of anhydrous aluminum sesquichlorohydrate in the Aluminum Sesquichlorohydrate Polyethylene Glycol by the formula:

$$Al[(26.98x + [17.01(3x - 1)]) + 35.453] / 26.98x$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Sesquichlorohydrate Propylene Glycol

$Al_2(OH)_3 \cdot 2Cl_2 \cdot nH_2O \cdot mC_3H_8O_2$

Aluminum chlorohydroxide propylene glycol complex.  
Aluminum hydroxychloride propylene glycol complex.

» Aluminum Sesquichlorohydrate Propylene Glycol consists of aluminum sesquichlorohydrate in which some of the waters of hydration have been replaced by propylene glycol. It encompasses a range of aluminum-to-chloride atomic ratios between 1.26:1 and 1.90:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum sesquichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum sesquichlorohydrate.

**Identification—**

**A**: A solution (1 in 10) responds to the tests for *Aluminum* (191) and for *Chloride* (191).

**B: Infrared Absorption (197F)—**

**Test specimen**—Dissolve 0.5 g in about 40 mL of water, and while mixing adjust with 2.5 N sodium hydroxide to a pH of  $9.55 \pm 0.05$ . Filter the suspension of precipitate thus obtained. Evaporate about 15 mL of the filtrate to about 1 mL on a hot plate. Deposit this solution on a silver chloride disk.

**Standard specimen**: a similar preparation of propylene glycol.

**pH (791)**: between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I (211)**: 2 µg per g.

**Delete the following:**

• **Heavy metals, Method I (231)**: 20 µg per g. (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Sesquichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Chlorohydrate*. The limit is 150 µg per g.

**Content of aluminum**—Using Aluminum Sesquichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Content of chloride**—Using Aluminum Sesquichlorohydrate Propylene Glycol instead of Aluminum Chlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Chlorohydrate*. Use the result obtained to calculate the *Aluminum/chloride atomic ratio*.

**Aluminum/chloride atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of chloride found in the test for *Content of chloride*, and multiply by 35.453/26.98, in which 35.453 and 26.98 are the atomic weights of chlorine and aluminum, respectively; the ratio is between 1.26:1 and 1.90:1.

**Assay**—Calculate the percentage of anhydrous aluminum sesquichlorohydrate in the Aluminum Sesquichlorohydrate Propylene Glycol by the formula:

$$Al[(26.98x + [17.01(3x - 1)]) + 35.453] / 26.98x$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *x* is the aluminum/chloride atomic ratio found in the test for *Aluminum/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Subacetate Topical Solution

» Aluminum Subacetate Topical Solution yields, from each 100 mL, not less than 2.30 g and not more than 2.60 g of aluminum oxide ( $Al_2O_3$ ), and not less than 5.43 g and not more than 6.13 g of acetic acid ( $C_2H_4O_2$ ). It may be stabilized by the addition of not more than 0.9 percent of boric acid.



Aluminum Subacetate Topical Solution may be prepared as follows.

Aluminum Sulfate . . . . .	145 g
Acetic Acid . . . . .	160 mL
Calcium Carbonate . . . . .	70 g
Purified Water, a sufficient quantity, to make . . . . .	1000 mL

Dissolve the Aluminum Sulfate in 600 mL of cold water, filter the solution, and add the Calcium Carbonate gradually, in several portions, with constant stirring. Then slowly add the Acetic Acid, mix, and set the mixture aside for 24 hours. Filter the product with the aid of vacuum if necessary, returning the first portion of the filtrate to the funnel. Wash the magma on the filter with small portions of cold water, until the total filtrate measures 1000 mL.

**Packaging and storage**—Preserve in tight containers.

**Identification**—It responds to the tests for *Aluminum* (191) and for the ferric chloride test for *Acetate* (191) with a deep red color upon the addition of ferric chloride TS. This color is destroyed by the addition of a mineral acid.

**pH** (791): between 3.8 and 4.6.

**Limit of boric acid**—Proceed as directed in the test for *Limit of boric acid* under *Aluminum Acetate Topical Solution*.

**Assay for aluminum oxide**—

*Edetate disodium titrant*—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

*Procedure*—Pipet 20 mL of Topical Solution into a 250-mL volumetric flask, add 5 mL of hydrochloric acid, dilute with water to volume, and mix. Pipet 25 mL of this dilution into a 250-mL beaker, and proceed as directed for *Procedure* in the *Assay for aluminum oxide* under *Aluminum Acetate Topical Solution*, beginning with "add, in the order named." Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 2.549 mg of  $\text{Al}_2\text{O}_3$ .

**Assay for acetic acid**—Proceed as directed in the *Assay for acetic acid* under *Aluminum Acetate Topical Solution*.

## Aluminum Sulfate

$\text{Al}_2(\text{SO}_4)_3 \cdot x\text{H}_2\text{O}$  (anhydrous) 342.15  
Sulfuric acid, aluminum salt (3:2), hydrate.  
Aluminum sulfate (2:3) hydrate [17927-65-0].  
Anhydrous 342.16 [10043-01-3].

» Aluminum Sulfate contains not less than 54.0 percent and not more than 59.0 percent of  $\text{Al}_2(\text{SO}_4)_3$ . It contains a varying amount of water of crystallization.

**Packaging and storage**—Preserve in well-closed containers.

**Identification**—A solution (1 in 10) responds to the tests for *Aluminum* and for *Sulfate* (191).

**pH** (791): not less than 2.9, in a solution (1 in 20).

**Water Determination, Method I** (921): not less than 41.0% and not more than 46.0%.

**Delete the following:**

• **Heavy metals** (231)—Dissolve 1.0 g in 2 mL of 1 N acetic acid, and dilute with water to 25 mL. The limit is 20 µg per g. (Official 1-Jan-2018)

**Limit of alkalies and alkaline earths**—To a boiling solution of 1.0 g in 150 mL of water add a few drops of methyl red TS and then add 6 N ammonium hydroxide just until the color of the solution changes to a distinct yellow. Add hot water to restore the volume to 150 mL, and filter while hot. Evaporate 75 mL of the filtrate to dryness, and ignite to constant weight: not more than 2 mg of residue remains (0.4%).

**Limit of ammonium salts**—Heat 1 g with 10 mL of 1 N sodium hydroxide on a steam bath for 1 minute: the odor of ammonia is not perceptible.

**Iron**—To 20 mL of a solution (1 in 150) add 0.3 mL of potassium ferrocyanide TS: no blue color is produced immediately.

**Assay**—

*Edetate disodium titrant*—Prepare and standardize as directed in the *Assay* under *Ammonium Alum*.

*Procedure*—Transfer about 7.5 g of Aluminum Sulfate, accurately weighed, to a 250-mL volumetric flask, and dissolve in water. Dilute with water to volume, mix, and pipet 10 mL of the solution into a 250-mL beaker. Proceed as directed in the *Assay for aluminum oxide* under *Aluminum Acetate Topical Solution*, beginning with "add, in the order named." Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 8.554 mg of  $\text{Al}_2(\text{SO}_4)_3$ .

## Aluminum Sulfate and Calcium Acetate for Topical Solution

### DEFINITION

Aluminum Sulfate and Calcium Acetate for Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum sulfate tetradecahydrate [ $\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}$ ] and calcium acetate monohydrate ( $\text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O}$ ).

### IDENTIFICATION

#### • A.

**Sample:** 0.25 g of Aluminum Sulfate and Calcium Acetate for Topical Solution

**Analysis:** Place the *Sample* in a test tube. Add 10 mL of water and 0.25 g of calcium carbonate. Heat on a steam bath for 10 min, and filter. Add 3–4 drops of ferric chloride TS to the filtrate. [NOTE—After the addition of the ferric chloride TS, the solution may be heated for 1 min to speed the reaction.]

**Acceptance criteria:** A reddish-brown color or precipitate indicates acetate.

#### • B. IDENTIFICATION TESTS—GENERAL, Sulfate (191) and Calcium (191)

**Sample solution:** Suspend 2 g of sample in 50 mL of water and filter.

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • ALUMINUM SULFATE TETRADECAHYDRATE

**Sample solution:** Transfer 10 g of Aluminum Sulfate and Calcium Acetate for Topical Solution to a 1000-mL volumetric flask. Add 100 mL of 1.2 N hydrochloric acid and 250 mL of water. Heat on a steam bath or hot plate until dissolved. Cool, and dilute with water to volume. Retain a portion of the *Sample solution* for the *Assay for Calcium Acetate Monohydrate*.



Blank: Water

Titrimetric system

Mode: Residual titration

Titrant: 0.02 M zinc sulfate VS

Endpoint detection: Visual

**Analysis:** Transfer a 5.0-mL aliquot of the *Sample solution* to a 250-mL conical flask. Add, in the order named, 40.0 mL of 0.01 M edetate disodium VS and 20 mL of acetic acid–ammonium acetate buffer TS, and mix by swirling. Add 50 mL of alcohol and 2 mL of dithizone TS, and titrate the excess 0.01 M edetate disodium VS with *Titrant* until the color changes from green-violet to a clear rose-pink. Perform a blank titration, substituting 5.0 mL of water for the *Sample solution*.

Calculate the percentage of aluminum sulfate tetradecahydrate  $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$  in the portion of Aluminum Sulfate and Calcium Acetate for Topical Solution taken:

$$\text{Result} = \{[D \times (V_B - V_S) \times M \times F] / W\} \times 100$$

$D$  = dilution factor, 1000/5.0

$V_B$  = blank titration volume (mL)

$V_S$  = sample titration volume (mL)

$M$  = molarity of the *Titrant* (mmol/mL)

$F$  = equivalency factor, 297.2 (mg/mmol)

$W$  = weight of sample used (mg)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of aluminum sulfate tetradecahydrate  $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$

#### • CALCIUM ACETATE MONOHYDRATE

**Sample:** Transfer a 5.0-mL aliquot of the *Sample solution* retained from the Assay for Aluminum Sulfate Tetradecahydrate to a 250-mL conical flask.

Titrimetric system

Mode: Direct titration

Titrant: 0.01 M edetate disodium VS

Endpoint detection: Visual

**Analysis:** Add 1–2 mL of 50% triethanolamine to mask the aluminum, and mix well. With constant stirring, add to the *Sample*, in the order named, 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue, and titrate with *Titrant*. The indicator will change from purple to a clear blue color at the endpoint.

Calculate the percentage of calcium acetate monohydrate  $(\text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O})$  in the portion of Aluminum Sulfate and Calcium Acetate for Topical Solution taken:

$$\text{Result} = [(D \times V \times M \times F) / W] \times 100$$

$D$  = dilution factor, 1000/5.0

$V$  = sample titration volume (mL)

$M$  = molarity of the *Titrant* (mmol/mL)

$F$  = equivalency factor, 176.2 (mg/mmol)

$W$  = weight of sample used (mg)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of calcium acetate monohydrate  $(\text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O})$

#### SPECIFIC TESTS

##### • pH (791)

**Sample solution:** 1 g of Aluminum Sulfate and Calcium Acetate for Topical Solution in 200 mL of water

**Acceptance criteria:** 4.0–4.8

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-unit containers, and protect from excessive heat.

## Aluminum Sulfate and Calcium Acetate Tablets for Topical Solution

### DEFINITION

Aluminum Sulfate and Calcium Acetate Tablets for Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amounts of aluminum sulfate tetradecahydrate  $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$  and calcium acetate monohydrate  $(\text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O})$ .

### IDENTIFICATION

#### • A. IDENTIFICATION TESTS—GENERAL, Aluminum (191)

**Sample solution:** Suspend 2 g of ground Tablet powder in 50 mL of water, and filter. Use a portion in the *Analysis*, and retain the remaining filtrate for *Identification* test B.

**Analysis:** Mix 2 mL of the *Sample solution* with 2 mL of water and 2 drops of 3 N hydrochloric acid.

**Acceptance criteria:** Meets the requirements of the ammonium hydroxide test

#### • B. IDENTIFICATION TESTS—GENERAL, Sulfate (191) and Calcium (191)

**Sample solution:** A portion of the filtrate retained from *Identification* test A

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • ALUMINUM SULFATE TETRADECAHYDRATE

**Sample solution:** Finely powder and mix NLT 20 Tablets. Weigh a portion of the powder, equivalent to 2.8 g of aluminum sulfate, and transfer to a 1000-mL volumetric flask. Add 100 mL of 1.2 N hydrochloric acid and 100 mL of water, and heat on a steam bath, with occasional swirling, to dissolve the powder. Allow the solution to cool, and dilute with water to volume. Retain a portion of the *Sample solution* for the Assay for Calcium Acetate Monohydrate.

Blank: Water

Titrimetric system

Mode: Residual titration

Titrant: 0.02 M zinc sulfate VS

Back-titrant: 0.01 M edetate disodium VS

Endpoint detection: Visual

**Analysis:** Transfer 25.0 mL of the *Sample solution* to a 250-mL conical flask. Add, in the order named, 40.0 mL of *Back-titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and mix by swirling. Add 50 mL of alcohol and 2 mL of dithizone TS, and titrate the excess *Back-titrant* with *Titrant* until the color changes from green-violet to a clear rose-pink. Perform a blank determination. Each mL of 0.01 M edetate disodium is equivalent to 2.972 mg of the labeled amount of aluminum sulfate tetradecahydrate  $[\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}]$ .

**Acceptance criteria:** 90.0%–110.0%

#### • CALCIUM ACETATE MONOHYDRATE

**Sample:** Transfer 20.0 mL of the *Sample solution* retained from the Assay for Aluminum Sulfate Tetradecahydrate to a 125-mL conical flask.

Titrimetric system

Mode: Direct titration

Titrant: 0.01 M edetate disodium VS

Endpoint detection: Visual

**Analysis:** With constant stirring, add to the *Sample*, in the order named, 0.5 mL of triethylamine, 10 mL of ammonia–ammonium chloride buffer TS, and 3 drops of a solution prepared by dissolving 500 mg of eriochrome black T titration in 10 mL of methanol. Titrate with *Titrant* to a violet endpoint. Each mL of 0.01 M edetate disodium is equivalent to 1.762 mg of the labeled amount of calcium acetate monohydrate  $(\text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O})$ .



Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

- **DISINTEGRATION** (701): 10 min
- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements for *Weight Variation*

#### SPECIFIC TESTS

- **pH** (791)  
Sample solution: 2 g of ground Tablet powder in 500 mL of water  
Acceptance criteria: 4.0–4.8
- **LOSS ON DRYING** (731)  
Analysis: Dry ground Tablet powder at 150° for 15 min.  
Acceptance criteria: NMT 18%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid excessive heat.

### Aluminum Zirconium Octachlorohydrate

$\text{Al}_y\text{Zr}(\text{OH})_{3y+4-x}\text{Cl}_x \cdot n\text{H}_2\text{O}$

» Aluminum Zirconium Octachlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium octachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum zirconium octachlorohydrate.

**Identification**—A solution (1 in 10) responds to the test for *Chloride* (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic**, *Method I* (211): 2 µg per g.

#### Delete the following:

• **Heavy metals**, *Method I* (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 *Acetate Buffer*, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of

each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found. (Official 1-Jan-2018)

#### Limit of iron—

**Standard preparation**—Transfer 2.0 mL of *Standard Iron Solution*, prepared as directed under *Iron* (241), to a 50-mL beaker.

**Test preparation**—Transfer 2.7 g of Aluminum Zirconium Octachlorohydrate to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL beaker.

**Procedure**—To each of the beakers containing the *Standard preparation* and the *Test preparation* add 5 mL of 6 N nitric acid, cover with a watch glass, and boil on a hot plate for 3 to 5 minutes. Allow to cool, add 5 mL of *Ammonium Thiocyanate Solution*, prepared as directed under *Iron* (241), transfer to separate 50-mL color comparison tubes, dilute with water to volume, and mix: the color of the solution from the *Test preparation* is not darker than that of the solution from the *Standard preparation* (150 µg per g).

**Content of aluminum**—Transfer about 0.15 g of Aluminum Zirconium Octachlorohydrate, accurately weighed, to a 150-mL beaker, and add 5 mL of water and 15 mL of hydrochloric acid. Heat this solution to boiling, and continue boiling for 5 minutes. Add 40 mL of water and 15.0 mL of 0.1 M edetate disodium VS. Heat the solution to boiling, and continue boiling for 5 minutes. Allow the solution to cool, add 10 to 15 mL of acetic acid–ammonium acetate buffer TS, and adjust with ammonium hydroxide to a pH of  $4.5 \pm 0.1$ . Add 20 mL of alcohol, and adjust with ammonium hydroxide to a pH of  $4.6 \pm 0.1$ . Add 5 to 10 drops of dithizone TS, and titrate with 0.1 M zinc sulfate VS until the first permanent purple-pink color appears. Perform a blank determination, and make any necessary correction. Calculate the percentage of aluminum (Al) in the Aluminum Zirconium Octachlorohydrate by the formula:

$$2.698[15.0 M_e - (zM_z + Z_e)] / W$$

in which  $M_e$  is the molarity of the edetate disodium VS;  $z$  is the volume, in mL, of zinc sulfate VS consumed;  $M_z$  is the molarity of the zinc sulfate VS;  $W$  is the quantity, in g, of Aluminum Zirconium Octachlorohydrate taken;  $Z_e$  is the equivalent volume, in mL, of edetate disodium VS consumed by the zirconium moiety, calculated as follows:

$$(\text{Zr}/M_e)(W/92.97)$$

in which  $Zr$  is the percentage of zirconium as determined in the test for *Content of zirconium*, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and the other terms are as defined above. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Transfer about 250 mg of Aluminum Zirconium Octachlorohydrate, accurately weighed, to a 150-mL beaker, and add 5 mL of water and 15 mL of hydrochloric acid. Heat this solution to boiling, and continue boiling for 6 to 8 minutes. Add 30 to 40 mL of water and 5 mL of hydrochloric acid, and heat to boiling. Add 1 drop of xylol orange TS, and, while still hot, titrate with 0.1 M edetate disodium VS until the color of the solution changes from pink to yellow. Perform a blank determination, and



make any necessary correction. Each mL of 0.1 M edetate disodium is equivalent to 9.297 mg of zirconium (Zr). Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Transfer about 250 mg of Aluminum Zirconium Octachlorohydrate, accurately weighed, to a 250-mL beaker, add 100 to 120 mL of water and 20 mL of diluted nitric acid, and swirl to dissolve. Titrate with 0.05 N silver nitrate VS using a calomel electrode and a silver billet electrode system, determining the endpoint potentiometrically. Each mL of 0.05 N silver nitrate is equivalent to 1.773 mg of chloride (Cl). Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)] / (Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Aluminum Zirconium Octachlorohydrate by the formula:

$$Al\{(26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1) / z\} / 26.98y\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Octachlorohydrate Solution

» Aluminum Zirconium Octachlorohydrate Solution consists of complex basic aluminum chloride that is polymeric and encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium octachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium octachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium octachlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211): Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found. (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Zirconium Octachlorohydrate Solution instead of Aluminum Chlorohydrate Solution, proceed as directed in the test for the *Limit of iron* under *Aluminum Chlorohydrate Solution*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Octachlorohydrate Solution, accurately weighed,



instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Octachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Octachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the *(aluminum plus zirconium)/chloride atomic ratio* by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Solution by the formula:

$$Al[(26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z]) + 35.453(y + 1)/z]/26.98y]$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the *(aluminum plus zirconium)/chloride atomic ratio* found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Octachlorohydrate Gly

» Aluminum Zirconium Octachlorohydrate Gly is a derivative of Aluminum Zirconium Octachlorohydrate in which some of the water molecules have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than 90.0 percent and not more than 110.0 per-

cent of the labeled amount of anhydrous aluminum zirconium octachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium octachlorohydrate.

### Identification—

**A:** A solution (1 in 10) responds to the test for *Chloride* (191).

**B:** Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found. (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Zirconium Octachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Octachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using Aluminum Zirconium Octachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium*



atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using Aluminum Zirconium Octachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Aluminum Zirconium Octachlorohydrate Gly by the formula:

$$Al/[26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z]/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Octachlorohydrate Gly Solution

» Aluminum Zirconium Octachlorohydrate Gly Solution is a solution of Aluminum Zirconium Octachlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium octachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium octachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium octachlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR absorption spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**D:** *Identification of glycine*—Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found. (Official 1-Jan-2018)

**Limit of iron**—Using about 5.4 g of Aluminum Zirconium Octachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as di-



rected in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Octachlorohydrate Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Octachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Octachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium octachlorohydrate in the Solution by the formula:

$$AK\{[26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z]] / 35.453(y + 1)/z\} / 26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Pentachlorohydrate

$Al_yZr_x(OH)_{3y+4-x}Cl_x \cdot nH_2O$

» Aluminum Zirconium Pentachlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1, and a range of (aluminum plus zirconium)-to-chloride atomic ratios

between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium pentachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum zirconium pentachlorohydrate.

**Identification**—A solution (1 in 10) responds to the test for *Chloride* (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found. • (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is



the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using Aluminum Zirconium Pentachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride, as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Aluminum Zirconium Pentachlorohydrate by the formula:

$$Al\{[26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z]] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for (Aluminum plus zirconium)/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Pentachlorohydrate Solution

» Aluminum Zirconium Pentachlorohydrate Solution is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium pentachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium pentachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium pentachlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solu-

tion, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

### Delete the following:

**Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found. (Official 1-Jan-2018)

**Limit of iron**—Using about 5.4 g of Aluminum Zirconium Pentachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Pentachlorohydrate Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Pentachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use



the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum; the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Pentachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the *(aluminum plus zirconium)/chloride atomic ratio* by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine; the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Solution by the formula:

$$Al/[(26.98y + 92.97 + (17.01)(3y + 4 - (y + 1)/z) + 35.453(y + 1)/z]/26.98y\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the *(aluminum plus zirconium)/chloride atomic ratio* found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Pentachlorohydrate Gly

» Aluminum Zirconium Pentachlorohydrate Gly is a derivative of Aluminum Zirconium Pentachlorohydrate in which some of the water molecules have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 6.0:1 and 10.0:1 and a range of *(aluminum plus zirconium)-to-chloride* atomic ratios between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium pentachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium pentachlorohydrate.

### Identification—

**A:** A solution (1 in 10) responds to the test for *Chloride* (191).

**B:** Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found. • (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Zirconium Pentachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Pentachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using Aluminum Zirconium Pentachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content*



of zirconium, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using Aluminum Zirconium Pentachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Aluminum Zirconium Pentachlorohydrate Gly by the formula:

$$Al\{[26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z]] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for (Aluminum plus zirconium)/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Pentachlorohydrate Gly Solution

» Aluminum Zirconium Pentachlorohydrate Gly Solution is a solution of Aluminum Zirconium Pentachlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium ratios between 6.0:1 and 10.0:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium pentachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium pentachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium pentachlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**D:** *Identification of glycine*—Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found. • (Official 1-Jan-2018)

**Limit of iron**—Using about 5.4 g of Aluminum Zirconium Pentachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.



**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Pentachlorohydrate Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Pentachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 6.0:1 and 10.0:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Pentachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the *(aluminum plus zirconium)/chloride atomic ratio* by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium pentachlorohydrate in the Solution by the formula:

$$Al/[26.98y + 92.97 + (17.01)(3y + 4 - (y + 1)/z) + 35.453(y + 1)/z]/26.98y]$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the *(aluminum plus zirconium)/chloride atomic ratio* found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Tetrachlorohydrate

$Al_yZr(OH)_{3y+4-x}Cl_x \cdot nH_2O$

» Aluminum Zirconium Tetrachlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than

90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium tetrachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum zirconium tetrachlorohydrate.

**Identification**—A solution (1 in 10) responds to the test for *Chloride* (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found. (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium



content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using Aluminum Zirconium Tetrachlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Aluminum Zirconium Tetrachlorohydrate by the formula:

$$Al\{[26.98y + 92.97 + 17.01\{3y + 4 - (y + 1)/z\} + 35.453(y + 1)/z]/26.98y\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Tetrachlorohydrate Solution

» Aluminum Zirconium Tetrachlorohydrate Solution is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium tetrachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium tetrachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium tetrachlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a

silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

*Test Preparation*—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

*Monitor Preparation*—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

*Procedure*—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found. • (Official 1-Jan-2018)

**Limit of iron**—Using about 5.4 g of Aluminum Zirconium Tetrachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Tetrachlorohydrate Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Tetrachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.



**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium* and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Tetrachlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Solution by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z]\} + 35.453(y + 1)/z/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for (Aluminum plus zirconium)/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Tetrachlorohydrate Gly

» Aluminum Zirconium Tetrachlorohydrate Gly is a derivative of Aluminum Zirconium Tetrachlorohydrate in which some of the water molecules have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium tetrachlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium tetrachlorohydrate.

### Identification—

A: A solution (1 in 10) responds to the test for *Chloride* (191).

B: Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found. (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Zirconium Tetrachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Tetrachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Content of zirconium**—Using Aluminum Zirconium Tetrachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using Aluminum Zirconium Tetrachlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of*



chloride under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio—**Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay—**Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Aluminum Zirconium Tetrachlorohydrate Gly by the formula:

$$Al\{[26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z]/26.98y\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for (Aluminum plus zirconium)/chloride atomic ratio, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Tetrachlorohydrate Gly Solution

» Aluminum Zirconium Tetrachlorohydrate Gly Solution is a solution of Aluminum Zirconium Tetrachlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 1.5:1 and 0.9:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium tetrachlorohydrate.

**Packaging and storage—**Preserve in well-closed containers.

**Labeling—**Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium tetrachlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium tetrachlorohydrate per mL responds to the test for *Chloride* (191).

**B: Identification of propylene glycol** (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wave-

lengths as that of a similar preparation of a film of propylene glycol.

**C: Identification of dipropylene glycol** (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**D: Identification of glycine—**Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation—**Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation—**Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure—**To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found. • (Official 1-Jan-2018)

**Limit of iron—**Using about 5.4 g of Aluminum Zirconium Tetrachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum—**Using about 0.3 g of Aluminum Zirconium Tetrachlorohydrate Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Content of zirconium—**Using about 500 mg of Aluminum Zirconium Tetrachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of*



zirconium under Aluminum Zirconium Octachlorohydrate. Use the result to calculate the Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Tetrachlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under Aluminum Zirconium Octachlorohydrate. Use the result to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 1.5:1 and 0.9:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium tetrachlorohydrate in the Solution by the formula:

$$Al\{[26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z]] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Trichlorohydrate

$Al_3Zr(OH)_{3y+4-x}Cl_x \cdot nH_2O$

» Aluminum Zirconium Trichlorohydrate is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium trichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the content of anhydrous aluminum zirconium trichlorohydrate.

**Identification**—A solution (1 in 10) responds to the test for *Chloride* (191).

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications:

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found. (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under Aluminum Zirconium Octachlorohydrate. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under Aluminum Zirconium Octachlorohydrate. Use the result obtained to calculate the Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Content of zirconium**—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under Aluminum Zirconium Octachlorohydrate. Use the result obtained to calculate the Aluminum/zirconium atomic ratio and the (Aluminum plus zirconium)/chloride atomic ratio.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using Aluminum Zirconium Trichlorohydrate instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under Aluminum Zirconium Octachlorohydrate. Use the result obtained to calculate the (Aluminum plus zirconium)/chloride atomic ratio.



**(Aluminum plus zirconium)/chloride atomic ratio—**Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay—**Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Aluminum Zirconium Trichlorohydrate by the formula:

$$Al[(26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z]) / 35.453(y + 1)/z] / 26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

### Aluminum Zirconium Trichlorohydrate Solution

» Aluminum Zirconium Trichlorohydrate Solution is a polymeric, loosely hydrated complex of basic aluminum zirconium chloride that encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium trichlorohydrate.

**Packaging and storage—**Preserve in well-closed containers.

**Labeling—**Label Solution to state the solvent used and the claimed concentration of anhydrous aluminum zirconium trichlorohydrate.

#### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium trichlorohydrate per mL responds to the test for *Chloride* (191).

**B: Identification of propylene glycol** (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C: Identification of dipropylene glycol** (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wave-

lengths as that of a similar preparation of a film of dipropylene glycol.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

#### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation—**Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation—**Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure—**To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found. • (Official 1-Jan-2018)

**Limit of iron—**Using about 5.4 g of Aluminum Zirconium Trichlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum—**Using about 0.3 g of Aluminum Zirconium Trichlorohydrate Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium—**Using about 500 mg of Aluminum Zirconium Trichlorohydrate Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio—**Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium* and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride—**Using about 500 mg of Aluminum Zirconium Trichlorohydrate Solution, accurately weighed, in-



stead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the test for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Solution by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio as determined in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride ratio determined in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Trichlorohydrate Gly

» Aluminum Zirconium Trichlorohydrate Gly is a derivative of Aluminum Zirconium Trichlorohydrate in which some of the water molecules have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium atomic ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of anhydrous aluminum zirconium trichlorohydrate.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The label states the form of glycine used and the claimed content of anhydrous aluminum zirconium trichlorohydrate.

### Identification—

**A:** A solution (1 in 10) responds to the test for *Chloride* (191).

**B:** Place about 0.5 g of it in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution [15 in 100 (w/w)].

**Arsenic, Method I** (211): 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

**Test Preparation**—Prepare as directed in the chapter. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

**Monitor Preparation**—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

**Procedure**—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 20 µg per g is found. (Official 1-Jan-2018)

**Limit of iron**—Using Aluminum Zirconium Trichlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The specified result is obtained (150 µg per g limit).

**Content of aluminum**—Using Aluminum Zirconium Trichlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using Aluminum Zirconium Trichlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium*, and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using Aluminum Zirconium Trichlorohydrate Gly instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result obtained to calculate the *(Aluminum plus zirconium)/chloride atomic ratio*.



**(Aluminum plus zirconium)/chloride atomic ratio—** Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride as determined in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay—**Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Aluminum Zirconium Trichlorohydrate Gly by the formula:

$$Al\{[26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z]/26.98y\}$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio found in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio found in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Aluminum Zirconium Trichlorohydrate Gly Solution

» Aluminum Zirconium Trichlorohydrate Gly Solution is a solution of Aluminum Zirconium Trichlorohydrate in which some of the waters of hydration have been displaced by glycine, calcium glycinate, magnesium glycinate, potassium glycinate, sodium glycinate, or zinc glycinate. It encompasses a range of aluminum-to-zirconium ratios between 2.0:1 and 5.99:1 and a range of (aluminum plus zirconium)-to-chloride atomic ratios between 2.1:1 and 1.51:1. The following solvents may be used: water, propylene glycol, or dipropylene glycol. It contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled concentration of anhydrous aluminum zirconium trichlorohydrate.

**Packaging and storage—**Preserve in well-closed containers.

**Labeling—**Label Solution to state the solvent and form of glycine used and the claimed concentration of anhydrous aluminum zirconium trichlorohydrate.

### Identification—

**A:** A solution containing the equivalent of about 100 mg of anhydrous aluminum zirconium trichlorohydrate per mL responds to the test for *Chloride* (191).

**B:** *Identification of propylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solution, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of propylene glycol.

**C:** *Identification of dipropylene glycol* (where stated on the label)—Add about 10 mL of isopropyl alcohol to 2 g of Solu-

tion, mix, and filter. Evaporate the filtrate to about 1 mL on a steam bath: the IR spectrum of a film of this solution on a silver chloride disk exhibits maxima only at the same wavelengths as that of a similar preparation of a film of dipropylene glycol.

**D:** *Identification of glycine*—Place about 1 g of Solution in a 50-mL beaker, add about 20 mL of water, and swirl to dissolve. Heat to boiling on a hot plate, and add about 60 mg of ninhydrin: a deep violet color immediately develops.

**pH** (791): between 3.0 and 5.0, in a solution prepared by diluting 3 g of the Solution with water to obtain 10 mL.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* using an accurately weighed quantity of the Solution. The limit is 2 µg per g.

### Delete the following:

• **Heavy metals, Method I** (231)—Proceed as directed in the chapter, except to use the following modifications.

*Test Preparation*—Prepare as directed in the chapter, using an accurately weighed quantity of Solution. If the solution is not clear after dilution to 40 mL, heat for several minutes between 60° and 80°, and cool to room temperature. If the solution remains cloudy, repeat from the beginning with the following modification: add 3 mL of hydrochloric acid prior to adjustment of the pH with 6 N ammonium hydroxide.

*Monitor Preparation*—Prepare as directed in the chapter, using the same modifications described for *Test Preparation* if necessary.

*Procedure*—To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of pH 3.5 Acetate Buffer, and heat gently to between 60° and 80°. To the *Standard Preparation* add 6 drops of sodium sulfide TS. To the *Test Preparation* and the *Monitor Preparation* add 12 drops of sodium sulfide TS. Cool to room temperature, and dilute the contents of each tube with water to 50 mL. Gently mix each tube by inverting twice. Allow to stand for 5 minutes, and view downward over a white surface. The color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the color of the solution from the *Monitor Preparation* is the same as or darker than the color of the solution from the *Standard Preparation*. If the color of the solution from the *Monitor Preparation* is lighter than the color of the solution from the *Standard Preparation*, repeat the procedure with the following modification: after the heating step, to the *Monitor Preparation* and the *Test Preparation* add 1.0 mL, instead of 12 drops, of sodium sulfide TS. Not more than 10 µg per g is found. (Official 1-Jan-2018)

**Limit of iron**—Using about 5.4 g of Aluminum Zirconium Trichlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Limit of iron* under *Aluminum Zirconium Octachlorohydrate*. The limit is 75 µg per g.

**Content of aluminum**—Using about 0.3 g of Aluminum Zirconium Trichlorohydrate Gly Solution instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of aluminum* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/chloride atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.

**Content of zirconium**—Using about 500 mg of Aluminum Zirconium Trichlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of zirconium* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the *Aluminum/zirconium atomic ratio* and the *(Aluminum plus zirconium)/chloride atomic ratio*.



**Aluminum/zirconium atomic ratio**—Divide the percentage of aluminum found in the test for *Content of aluminum* by the percentage of zirconium found in the test for *Content of zirconium* and multiply by 92.97/26.98, in which 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, and 26.98 is the atomic weight of aluminum: the ratio is between 2.0:1 and 5.99:1.

**Content of chloride**—Using about 500 mg of Aluminum Zirconium Trichlorohydrate Gly Solution, accurately weighed, instead of Aluminum Zirconium Octachlorohydrate, proceed as directed in the test for the *Content of chloride* under *Aluminum Zirconium Octachlorohydrate*. Use the result to calculate the (Aluminum plus zirconium)/chloride atomic ratio.

**(Aluminum plus zirconium)/chloride atomic ratio**—Calculate the (aluminum plus zirconium)/chloride atomic ratio by the formula:

$$[(Al/26.98) + (Zr/92.97)]/(Cl/35.453)$$

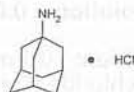
in which *Al*, *Zr*, and *Cl* are the percentages of aluminum, zirconium, and chloride found in the tests for *Content of aluminum*, *Content of zirconium*, and *Content of chloride*, respectively; 26.98 is the atomic weight of aluminum; 92.97 is the atomic weight of zirconium corrected for 2% hafnium content; and 35.453 is the atomic weight of chlorine: the ratio is between 2.1:1 and 1.51:1.

**Assay**—Calculate the percentage of anhydrous aluminum zirconium trichlorohydrate in the Solution by the formula:

$$Al\{26.98y + 92.97 + 17.01[3y + 4 - (y + 1)/z] + 35.453(y + 1)/z\}/26.98y$$

in which *Al* is the percentage of aluminum found in the test for *Content of aluminum*, *y* is the aluminum/zirconium atomic ratio, as determined in the test for *Aluminum/zirconium atomic ratio*, *z* is the (aluminum plus zirconium)/chloride atomic ratio as determined in the test for *(Aluminum plus zirconium)/chloride atomic ratio*, 26.98 is the atomic weight of aluminum, 92.97 is the atomic weight of zirconium corrected for 2% hafnium content, 17.01 is the molecular weight of the hydroxide anion (OH), and 35.453 is the atomic weight of chlorine (Cl).

## Amantadine Hydrochloride



$C_{10}H_{17}N \cdot HCl$  187.71  
Tricyclo[3.3.1.1<sup>3,7</sup>]decan-1-amine, hydrochloride;  
1-Adamantanamine hydrochloride [665-66-7].

### DEFINITION

Amantadine Hydrochloride contains NLT 98.5% and NMT 101.5% of  $C_{10}H_{17}N \cdot HCl$ .

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197S)

Cell: 1 mm

**Sample solution:** 50 mg in 10 mL of 0.1 N hydrochloric acid, and filter. Transfer the filtrate to a suitable separator, add 1 mL of 5 N sodium hydroxide, and extract with 5 mL of methylene chloride.

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Sample:** Dissolve 120 mg of Amantadine Hydrochloride in a mixture of 30 mL of glacial acetic acid and 10 mL of mercuric acetate TS.

**Analysis:** Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using suitable electrodes. Perform a blank determination. Each mL of 0.1 N perchloric acid is equivalent to 18.77 mg of amantadine hydrochloride ( $C_{10}H_{17}N \cdot HCl$ ).

**Acceptance criteria:** 98.5%–101.5%

### IMPURITIES

**Delete the following:**

#### • HEAVY METALS, Method I (231)

**Test preparation:** Use 1 mL of 1 N acetic acid.

**Acceptance criteria:** NMT 10 ppm (Official: 1-Jan-2018)

#### • ORGANIC IMPURITIES

**Internal standard solution:** 50 mg/mL of adamantane in dichloromethane

**Standard solution:** Transfer 10 mg of USP Amantadine Hydrochloride RS to a separator. Add 20 mL of 5.0 N sodium hydroxide and 18 mL of dichloromethane, and shake for 10 min. Remove the water layer, dry the organic layer by swirling with anhydrous sodium sulfate, and allow to stand for a few min to ensure that all remaining water has been removed. Filter, collect the filtrate in a 20-mL volumetric flask, add 0.2 mL of *Internal standard solution*, and dilute with dichloromethane to volume.

**Sample solution:** Transfer 1.0 g of Amantadine Hydrochloride to a separator. Add 20 mL of 5.0 N sodium hydroxide and 18 mL of dichloromethane, and shake for 10 min. Remove the water layer, dry the organic layer by swirling with anhydrous sodium sulfate, and allow to stand for a few min to ensure that all remaining water has been removed. Filter, collect the filtrate in a 20-mL volumetric flask, add 0.2 mL of *Internal standard solution*, and dilute with dichloromethane to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Detector temperature:** 300°

**Column:** 0.53-mm × 30-m fused-silica column coated with 1.0-μm G27 stationary phase

**Column temperature:** See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	0	70	5
70	10	250	At least 17

**Carrier gas:** Helium

**Flow rate:** 4 mL/min

**Injection size:** 2 μL

**Injector temperature:** 220°

**Injection type:**

**Split flow:** 200 mL/min

**Split flow ratio:** 50:1

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for adamantane and amantadine are about 0.7 and 1.0, respectively.]



**Suitability requirements**

**Resolution:** NLT 20 between adamantane and amantadine

**Relative standard deviation:** NMT 5.0% determined from the peak response ratios of amantadine to adamantane

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of amantadine hydrochloride ( $C_{10}H_{17}N \cdot HCl$ ) taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of each impurity to adamantane from the *Sample solution*

$R_S$  = peak response ratio of amantadine to adamantane from the *Standard solution*

$C_S$  = concentration of USP Amantadine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** NMT 0.3%

**Total impurities:** NMT 1.0%

**SPECIFIC TESTS**• **PH (791)**

**Sample:** 0.2 g/mL in water

**Acceptance criteria:** 3.0–5.5

• **CLARITY AND COLOR OF SOLUTION**

**Sample:** Dissolve 2 g in 10 mL of water.

**Acceptance criteria:** Solution is clear and nearly colorless.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**

USP Amantadine Hydrochloride RS

**Amantadine Hydrochloride Capsules****DEFINITION**

Amantadine Hydrochloride Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of amantadine hydrochloride ( $C_{10}H_{17}N \cdot HCl$ ).

**IDENTIFICATION**• **INFRARED ABSORPTION (197S)**

**Cell:** 1 mm

**Sample solution:** Place the contents of Capsules, equivalent to 200 mg of amantadine hydrochloride, in a vessel, dissolve in 0.1 N hydrochloric acid, and filter. Transfer the filtrate to a separator, add 1 mL of 5 N sodium hydroxide, and extract with 5 mL of methylene chloride. Filter the extract through anhydrous sodium sulfate, and rinse the anhydrous sodium sulfate with 2 mL of methylene chloride.

**ASSAY**• **PROCEDURE**

**Internal standard solution:** 0.4 mg/mL of naphthalene in hexane

**Standard stock solution:** 2 mg/mL of USP Amantadine Hydrochloride RS in water

**Standard solution:** Pipet 25.0 mL of *Standard stock solution* into a 250-mL separator, and add 25 mL of 2.0 N sodium hydroxide and 50.0 mL of *Internal standard solution*. Shake for 60 min, and collect the hexane layer (*Standard solution*).

**Sample solution:** Transfer NLT 20 Capsules to a 200-mL volumetric flask. Add 40 mL of 0.1 N hydrochloric acid, and heat gently to achieve complete dissolution. Cool, and dilute with water to volume. Pipet 5.0 mL of the solution into a 250-mL separator, and add 40 mL of 1.0 N sodium hydroxide and 50.0 mL of *Internal standard solution*. Shake for 60 min, and collect the hexane layer (*Sample solution*).

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 2-mm  $\times$  1.22-m; glass column packed with 10% phase G1 on 100- to 120-mesh support S1A

**Temperature**

**Column:** 115°

**Injector:** 250°

**Detector block:** 250°

**Injection size:** 1  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2 between naphthalene and amantadine

**Tailing factor:** NMT 2.0 for the analyte peak

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{10}H_{17}N \cdot HCl$  in the portion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratios from the *Sample solution*

$R_S$  = peak response ratios from the *Standard solution*

$C_S$  = concentration of USP Amantadine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0%

**PERFORMANCE TESTS**• **DISSOLUTION (711)**

**Test 1:** Procedure for a Pooled Sample

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Internal standard solution:** 0.054 mg/mL of naphthalene in hexane

**Standard stock solution:** 0.1 mg/mL of USP Amantadine Hydrochloride RS in water

**Standard solution:** Pipet 15.0 mL of *Standard stock solution* into a 50-mL screw-capped test tube, add 5.0 mL of 5 N sodium hydroxide and 10.0 mL of *Internal standard solution*, and shake for 60 min. Collect the hexane layer.

**Sample solution:** Filter 15.0 mL of the solution under test, and place into a 50-mL screw-capped test tube. Pipet 5.0 mL of 5 N sodium hydroxide and 10.0 mL of the *Internal standard solution* into the test tube, and shake for 60 min. Collect the hexane layer (*Sample solution*).

**Chromatographic system:** Proceed as directed in the *Assay*.

**Injection size:** 2.5  $\mu$ L

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** NLT 75% (Q) of the labeled amount of  $C_{10}H_{17}N \cdot HCl$  is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.



**Medium:** Water; 900 mL

**Apparatus 2:** 75 rpm, with sinkers. [NOTE—A suitable sinker is available as catalog number CAPWHT-2S from [www.qia-lc.com](http://www.qia-lc.com) or [www.tabletdissolution.com](http://www.tabletdissolution.com) or [www.labhut.com](http://www.labhut.com).]

**Time:** 45 min

**Internal standard solution:** 0.06 mg/mL of naphthalene in hexanes

**Standard stock solution:** 0.12 mg/mL of USP Amantadine Hydrochloride RS in *Medium*

**Standard solution:** Transfer 60.0 mL of the *Standard stock solution* to a 200-mL volumetric flask. Add 20 mL of 5 N sodium hydroxide and 40.0 mL of *Internal standard solution*. Shake the flask for approximately 10 min, and allow the layers to separate. Use the top layer for injection. The final concentration is about 0.18 mg/mL.

**Sample solution:** Transfer 3.0 mL of the solution under test to a centrifuge tube. Add 1.0 mL of 5 N sodium hydroxide and 2.0 mL of *Internal standard solution*. Shake the tube for approximately 10 min, and allow the layers to separate. Use the top layer for injection.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.32-mm × 30-m, 0.25-μm film, phase G1

**Temperature**

**Oven:** 100° for 3 min, to 200° at 10°/min, held at 200° for 2 min

**Injector:** 250°

**Detector:** 300°

**Carrier gas:** Helium, 1.4 mL/min

**Flow rate:** 20 mL/min

**Injection size:** 2 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2 between naphthalene and amantadine hydrochloride

**Tailing factor:** NMT 2.0 for amantadine hydrochloride

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amantadine hydrochloride released:

$$\text{Result} = (R_U/R_S) \times (C_S/L) \times V \times 100$$

$R_U$  = ratio of the peak areas from the *Sample solution*

$R_S$  = average ratio of the peak areas from the *Standard solution*

$C_S$  = concentration of amantadine hydrochloride in the *Standard stock solution* (mg/mL)

$L$  = label claim (mg/capsule)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of amantadine hydrochloride is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

#### • USP REFERENCE STANDARDS (11)

USP Amantadine Hydrochloride RS

### Amantadine Hydrochloride Oral Solution

» Amantadine Hydrochloride Oral Solution contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of amantadine hydrochloride ( $C_{10}H_{17}N \cdot HCl$ ).

**Packaging and storage—**Preserve in tight containers.

#### USP Reference standards (11)—

USP Amantadine Hydrochloride RS

#### Identification, Infrared Absorption (197S)—

*Cell:* 1 mm.

**Solution—**Place a volume of Oral Solution, equivalent to about 200 mg of amantadine hydrochloride, in a vessel, dissolve in 0.1 N hydrochloric acid, and filter. Transfer the filtrate to a separator, add 10 mL of 0.5 N sodium hydroxide, and extract with 5 mL of methylene chloride. Filter the extract through anhydrous sodium sulfate, and rinse the anhydrous sodium sulfate with 2 mL of methylene chloride.

#### Assay—

**Internal standard solution, Standard preparation, and Chromatographic system—**Proceed as directed in the Assay under *Amantadine Hydrochloride Capsules*.

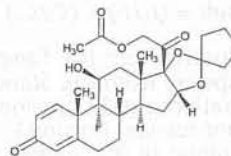
**Assay preparation—**Pipet 5.0 mL of the Oral Solution into a 250-mL conical flask, and add 45 mL of 1.0 N sodium hydroxide and 50.0 mL of *Internal standard solution*. Shake for 60 minutes, and collect the hexane layer (*Assay preparation*).

**Procedure—**Proceed as directed in the Assay under *Amantadine Hydrochloride Capsules*. Calculate the quantity, in mg, of amantadine hydrochloride ( $C_{10}H_{17}N \cdot HCl$ ) in the portion of Oral Solution taken by the formula:

$$50C(R_U/R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Amantadine Hydrochloride RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Amcinonide



$C_{28}H_{35}FO_7$

502.57

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-16,17-[cyclopentylidenebis(oxy)]-9-fluoro-11-hydroxy-, (11β, 16α)-;

9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with cyclopentanone, 21-acetate [51022-69-6].



**DEFINITION**

Amcinonide contains NLT 97.0% and NMT 102.0% of amcinonide ( $C_{28}H_{35}FO_7$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)  
Analytical wavelength: 238 nm  
Sample solution: 40 µg/mL in methanol  
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

**ASSAY**

- **PROCEDURE**  
Solution A: Acetonitrile and water (7:13)  
Solution B: Acetonitrile and water (7:3)  
System suitability solution: 12.5 µg/mL of butylparaben and 20 µg/mL of USP Amcinonide RS in Solution B  
Standard solution: 0.02 mg/mL of USP Amcinonide RS in Solution B  
Sample solution: 0.02 mg/mL of Amcinonide in Solution B. Sonicate for 5 min.  
Mobile phase: See Table 1. Equilibrate the system with Solution A.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2.5	100	0
10	0	100

**Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

**System suitability**

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for butylparaben and amcinonide are 0.78 and 1.0, respectively.]

**Suitability requirements**

Resolution: NLT 8.0 between butylparaben and amcinonide, System suitability solution

Tailing factor: NMT 1.5, Standard solution

Relative standard deviation: NMT 2.0%, Standard solution

**Analysis**

Samples: Standard solution and Sample solution

Calculate the percentage of amcinonide ( $C_{28}H_{35}FO_7$ ) in the portion of Amcinonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Amcinonide RS in the Standard solution (mg/mL)

$C_U$  = concentration of the Sample solution (mg/mL)

Acceptance criteria: 97.0%–102.0% on the dried basis

**IMPURITIES****Delete the following:**

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-Jan-2018)

**SPECIFIC TESTS**

- **OPTICAL ROTATION, Specific Rotation** (781S)  
Sample solution: 10 mg/mL in chloroform  
Acceptance criteria: +89.4° to +94.0°
- **LOSS ON DRYING** (731)  
Sample: Amcinonide  
Analysis: Dry the Sample at 105° for 4 h.  
Acceptance criteria: NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP Amcinonide RS

**Amcinonide Cream****DEFINITION**

Amcinonide Cream is Amcinonide in a suitable cream base. It contains NLT 90.0% and NMT 115.0% of the labeled amount of amcinonide ( $C_{28}H_{35}FO_7$ ).

**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 100 µg/mL of USP Amcinonide RS in chloroform

Sample solution: Place 2 g of Cream in a 150-mL beaker, add 50 mL of chloroform and 15 g of anhydrous sodium sulfate, and stir with a glass rod to dissolve the specimen. Filter the solution, and clarify the filtrate, if necessary, by the further addition of anhydrous sodium sulfate and a second filtration. Evaporate the filtrate to dryness, and dissolve the residue in chloroform to obtain a solution containing 100 µg/mL of amcinonide.

**Chromatographic system**

(See Chromatography (621), Thin-Layer Chromatography.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 25 µL

Developing solvent: Ether

Visualization: Short wavelength UV

**Analysis**

Samples: Standard solution and Sample solution

Apply the Samples on a line parallel to and 3 cm from the bottom edge of the plate. Develop the chromatogram until the solvent front has moved about 12 cm above the line of application.

Acceptance criteria: The intensity and the  $R_f$  value of the principal spot of the Sample solution are similar to those of the Standard solution.

**ASSAY**• **PROCEDURE**

Solution A: Acetonitrile and water (7:13)

Solution B: Acetonitrile and water (7:3)

Standard solution: 0.02 mg/mL of USP Amcinonide RS in Solution B

System suitability solution: 12.5 µg/mL of butylparaben and 20 µg/mL of USP Amcinonide RS in Solution B

Sample stock solution: 0.2 mg/mL of amcinonide prepared as follows. Transfer a quantity of Cream, equivalent to 10 mg of amcinonide, to a 50-mL volumetric flask. Add 5 mL of Solution B and 15 mL of acetonitrile, and heat over a steam bath until dissolved. Add 20 mL of Solution B while hot, cool to room temperature, dilute with Solution B to volume, and refrigerate for 30 min. Vigorously shake the solution to disperse the mixture, and filter while cold. Use the filtrate.



**Sample solution:** 0.02 mg/mL of amcinonide in *Solution B* from the *Standard stock solution*  
**Mobile phase:** See *Table 1*. Equilibrate the system with *Solution A*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
2.5	100	0
10	0	100

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for butylparaben and amcinonide are 0.78 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 8.0 between butylparaben and amcinonide, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amcinonide ( $C_{28}H_{35}FO_7$ ) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amcinonide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amcinonide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–115.0%

**PERFORMANCE TESTS**

- **MINIMUM FILL** (755): Meets the requirements

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **pH** (791): 3.5–5.2

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Amcinonide RS

**Amcinonide Ointment**

» Amcinonide Ointment is Amcinonide in a suitable ointment base. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of amcinonide ( $C_{28}H_{35}FO_7$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Amcinonide RS

**Identification**—It meets the requirements for the *Identification* test under *Amcinonide Cream*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**Minimum fill** (755): meets the requirements.

**Assay**—

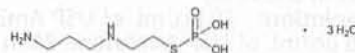
*Solution A*, *Solution B*, *Mobile phase*, *System suitability solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Amcinonide*, except to use a 240-nm detector.

**Assay preparation**—Dissolve an accurately weighed quantity of Ointment in a suitable volume of a mixture of acetonitrile and chloroform (4:1) by heating in a hot water bath, cooling, and adjusting quantitatively with the same solvent mixture to obtain a solution having a concentration of about 0.2 mg of amcinonide per mL. Cool to room temperature, dilute with acetonitrile to volume, and filter. Transfer 5 mL of this solution to a 50-mL volumetric flask, dilute with *Solution B* to volume, and mix.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of amcinonide ( $C_{28}H_{35}FO_7$ ) in the portion of Ointment taken by the formula:

$$500C(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Amcinonide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Amifostine**

$C_5H_{15}N_2O_3PS \cdot 3H_2O$  268.27

Ethanesithiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester), trihydrate;

S-[2-(3-Aminopropyl)amino]ethyl]dihydrogen phosphorothioate, trihydrate [112901-68-5].

**DEFINITION**

Amifostine contains NLT 78.0% and NMT 82.0% of  $C_5H_{15}N_2O_3PS$ , calculated on the as-is basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 0.94 g/L of sodium 1-hexanesulfonate. Adjust with phosphoric acid to a pH of 3.0.



**Mobile phase:** Methanol and Buffer (7:18)  
**Standard solution:** 3 mg/mL of USP Amifostine RS in water. [NOTE—Inject immediately after preparation.]  
**Sample solution:** 3 mg/mL of Amifostine in water. [NOTE—Inject immediately after preparation.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Autosampler temperature:** 4°

**Flow rate:** 1.0 mL/min

**Injection size:** 10 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Column efficiency:** NLT 1000 theoretical plates

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>5</sub>H<sub>15</sub>N<sub>2</sub>O<sub>3</sub>PS in the portion of Amifostine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amifostine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amifostine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 78.0%–82.0% on the as-is basis

**IMPURITIES****Inorganic Impurities**

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm (Official 1-Jan-2018)

**Organic Impurities****• PROCEDURE**

**Mobile phase and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 70 μg/mL of USP Amifostine Thiol RS and 16 μg/mL of USP Amifostine RS in water.

[NOTE—Inject immediately after preparation.]

**System suitability solution:** Use the *Standard solution* as described in the *Assay*. [NOTE—Inject immediately after preparation.]

**Sample solution:** 15 mg/mL of Amifostine in water. [NOTE—Inject immediately after preparation.]

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

**Suitability requirements**

**Column efficiency:** NLT 1000 theoretical plates, *System suitability solution*

**Tailing factor:** NMT 2.0, *System suitability solution*

**Relative standard deviation:** NMT 15.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amifostine thiol in the portion of Amifostine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of amifostine thiol from the *Sample solution*

$r_S$  = peak response of amifostine thiol from the *Standard solution*

$C_S$  = concentration of USP Amifostine Thiol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amifostine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of amifostine thiol, 134.24

$M_{r2}$  = molecular weight of amifostine thiol dihydrochloride, 207.17

Calculate the percentage of any other individual impurity in the portion of Amifostine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity in the *Sample solution*

$r_S$  = peak response of amifostine in the *Standard solution*

$C_S$  = concentration of USP Amifostine RS in the *Standard solution* (μg/mL)

$C_U$  = concentration of the *Sample solution* (μg/mL)

**Acceptance criteria**

**Amifostine thiol:** NMT 0.3%

**Any individual impurity, excluding amifostine thiol:** NMT 0.1%

**Total impurities including amifostine thiol:** NMT 0.3%

**SPECIFIC TESTS**

- **pH (791):** 6.5–7.5, in a solution (5 in 100)

- **WATER DETERMINATION**, *Method 1c* (921)

**Sample solution:** To 100.0 mg of Amifostine, contained in a stoppered centrifuge tube, add 10.0 mL of the solution of *N*-ethylmaleimide in methanol (4 in 100), and sonicate for 15 min. Shake to disperse, and sonicate for an additional 15 min. Use 1.0 mL of the supernatant.

**Acceptance criteria:** 19.2%–21.2%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store in a refrigerator.

- **USP REFERENCE STANDARDS (11)**

USP Amifostine RS

USP Amifostine Thiol RS

Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrochloride.

C<sub>5</sub>H<sub>16</sub>N<sub>2</sub>SCl<sub>2</sub> 207.17

**Amifostine for Injection****DEFINITION**

Amifostine for Injection is a sterile, crystalline substance suitable for parenteral use. It contains NLT 90.0% and NMT 110.0% of the labeled amount of amifostine (C<sub>5</sub>H<sub>15</sub>N<sub>2</sub>O<sub>3</sub>PS).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Buffer:** 0.94 g/L of sodium 1-hexanesulfonate. Adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Methanol and Buffer (7:18)

**Standard solution:** 3 mg/mL of USP Amifostine RS in water. [NOTE—Inject immediately after preparation, or refrigerate until use.]

**Sample solution:** 3 mg/mL of amifostine from Amifostine for Injection, in water. [NOTE—Inject immediately after preparation, or refrigerate until use.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Autosampler temperature: 4°

Flow rate: 1.0 mL/min

Injection size: 10 μL

System suitability

Sample: Standard solution

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of C<sub>5</sub>H<sub>15</sub>N<sub>2</sub>O<sub>3</sub>PS in the portion of Amifostine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak responses from the Sample solution

$r_S$  = peak responses from the Standard solution

$C_S$  = concentration of USP Amifostine RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of amifostine in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

## IMPURITIES

### Organic Impurities

#### • PROCEDURE 1

**Mobile phase and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution 1:** 70 μg/mL of USP Amifostine Thiol RS in water

**Standard solution 2:** 15 μg/mL of sodium thiophosphate and 13 μg/mL of N,N-dimethylformamide in water. [NOTE—The retention times of sodium thiophosphate and N,N-dimethylformamide are about 2 min and about 3.6 min, respectively.]

**Sample solution:** 2.4 mg/mL of amifostine from Amifostine for Injection in water. [NOTE—Inject immediately after preparation.]

**System suitability**

Samples: Standard solution 1 and Standard solution 2

Suitability requirements

Relative standard deviation: NMT 10.0%, Standard solution 1; NMT 4.0%, Standard solution 2

Analysis

Samples: Standard solution 1, Standard solution 2, and Sample solution

Calculate the percentage of amifostine thiol in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of amifostine thiol from the Sample solution

$r_S$  = peak response of amifostine thiol from Standard solution 1

$C_S$  = concentration of USP Amifostine Thiol RS in Standard solution 1 (mg/mL)

$C_U$  = concentration of amifostine in the Sample solution (mg/mL)

$M_{r1}$  = molecular weight of amifostine thiol, 134.24

$M_{r2}$  = molecular weight of amifostine thiol dihydrochloride, 207.17

Calculate the percentage of sodium thiophosphate or N,N-dimethylformamide in the portion of sample taken, if present:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of sodium thiophosphate or N,N-dimethylformamide from the Sample solution

$r_S$  = peak response of sodium thiophosphate or N,N-dimethylformamide from Standard solution 2

$C_S$  = concentration of sodium thiophosphate or N,N-dimethylformamide in Standard solution 2 (mg/mL)

$C_U$  = concentration of amifostine in the Sample solution (mg/mL)

Calculate the percentage of any other individual, unspecified impurity in the portion of sample taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each individual impurity in the Sample solution

$r_T$  = total of all peak responses in the Sample solution

**Acceptance criteria:** NMT 0.1% of sodium thiophosphate; NMT 0.088% of N,N-dimethylformamide; NMT 0.1% of any other individual unspecified impurity

#### • PROCEDURE 2

**Buffer:** 0.4 g/L of sodium 1-octanesulfonate. Adjust with trifluoroacetic acid to a pH of 2.5 ± 0.1.

**Mobile phase:** Acetonitrile and Buffer (1:3)

**Standard solution:** 46 μg/mL of USP Amifostine Disulfide RS in water

**Sample solution:** Dilute a quantity of Amifostine for Injection in water to prepare a solution equivalent to 10 mg/mL. [NOTE—Inject immediately after preparation.]

**Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 247 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Autosampler temperature: 4°

Flow rate: 1.0 mL/min

Injection size: 10 μL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 4.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of amifostine disulfide in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of amifostine disulfide from the Sample solution

$r_S$  = peak response of amifostine disulfide from the Standard solution

$C_S$  = concentration of USP Amifostine Disulfide RS in the Standard solution (mg/mL)

$C_U$  = concentration of amifostine in the Sample solution (mg/mL)

$M_{r1}$  = molecular weight of amifostine disulfide, 266.47

$M_{r2}$  = molecular weight of amifostine disulfide tetrahydrochloride, 412.31

**Acceptance criteria:** NMT 2.0% of total impurities, including amifostine thiol and amifostine disulfide

## SPECIFIC TESTS

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*. When constituted with 0.9% Sodium Chloride Injection, the solution must completely dissolve in 45 s.

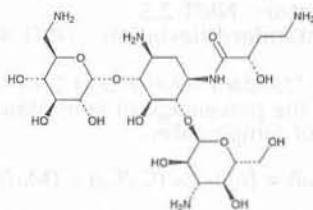


- **X-RAY DIFFRACTION (941):** Its X-ray diffraction pattern conforms to that of USP Amifostine RS, similarly determined.
- **STERILITY TESTS (71):** It meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined, Membrane Filtration*.
- **PH (791):** 6.5–7.5, in a solution constituted as directed in the labeling.
- **WATER DETERMINATION, Method 1c (921)**  
**Sample solution:** To 100.0 mg of Amifostine for Injection, contained in a stoppered centrifuge tube, add 10.0 mL of a solution of *N*-ethylmaleimide in methanol (4 in 100), and sonicate for 15 min. Shake to disperse, and sonicate for an additional 15 min. Use 1.0 mL of the supernatant.  
**Acceptance criteria:** 18.0%–22.0%
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST (85):** Contains NMT 0.2 USP Endotoxin Unit/mg of amifostine
- **OTHER REQUIREMENTS:** Meets the requirements for *Labeling (7), Labels and Labeling for Injectable Products*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements (659), Injection Packaging*, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
 USP Amifostine RS  
 USP Amifostine Disulfide RS  
 1,3-Propanediamine, *N,N*-(dithiodi-2,1-ethanediyl)bis, tetrahydrochloride.  
 $C_{10}H_{30}N_4S_2Cl_4$  412.32  
 USP Amifostine Thiol RS  
 Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrochloride.  
 $C_5H_{16}N_2S_2Cl_2$  207.17  
 USP Endotoxin RS

## Amikacin



$C_{22}H_{43}N_5O_{13}$  585.60  
 D-Streptamine, O-3-amino-3-deoxy- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 6)-O-[6-amino-6-deoxy- $\alpha$ -D-glucopyranosyl(1 $\rightarrow$ 4)]-N<sup>1</sup>-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-, (S)-;  
 O-3-Amino-3-deoxy- $\alpha$ -D-glucopyranosyl(1 $\rightarrow$ 4)-O-[6-amino-6-deoxy- $\alpha$ -D-glucopyranosyl(1 $\rightarrow$ 6)]-N<sup>3</sup>-(4-amino-2-hydroxybutyl)-2-deoxy-L-streptamine [37517-28-5].

**DEFINITION**

Amikacin has a potency of NLT 900  $\mu$ g/mg of  $C_{22}H_{43}N_5O_{13}$ , calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the peak for amikacin of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****PROCEDURE**

**Mobile phase:** 0.115 N sodium hydroxide  
**System suitability solution:** 0.02 mg/mL of USP Amikacin RS and 0.008 mg/mL of USP Kanamycin Sulfate RS in water  
**Standard solution:** 0.02 mg/mL of USP Amikacin RS in water  
**Sample solution:** 0.02 mg/mL of Amikacin in water  
**Chromatographic system**  
 (See *Chromatography (621), System Suitability*.)  
**Mode:** LC  
**Detector:** Electrochemical  
**Detector mode:** Integrated amperometric mode  
**Electrodes**  
**Working:** Gold  
**Reference:** Silver-silver chloride  
**Detector settings:** See Table 1.

Table 1

Potential		Time (ms)
No.	V	
E <sub>1</sub>	0.04	200
E <sub>2</sub>	0.8	190
E <sub>3</sub>	-0.8	190

**Columns**

**Guard:** Packing L47

**Analytical:** 4-mm  $\times$  25-cm; 10- $\mu$ m packing L47

**Flow rate:** 0.5 mL/min

**Injection size:** 20  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for kanamycin and amikacin are 0.8 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between kanamycin and amikacin, *System suitability solution*

**Tailing factor:** NMT 2, *Standard solution*

**Relative standard deviation:** NMT 3%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in  $\mu$ g, of amikacin ( $C_{22}H_{43}N_5O_{13}$ ) in each mg of Amikacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Amikacin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

$P$  = potency of amikacin in USP Amikacin RS (mg/mg)

$F$  = conversion factor, 1000  $\mu$ g/mg

**Acceptance criteria:** NLT 900  $\mu$ g/mg on the anhydrous basis

**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 1.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

**SPECIFIC TESTS**

- **OPTICAL ROTATION, Specific Rotation (781S)**

**Sample solution:** 20 mg/mL in water

**Acceptance criteria:** +97° to +105°







Table 3

Ratio of Amikacin:H <sub>2</sub> SO <sub>4</sub>	Acceptance Criteria
1:2	2.0–4.0
1:1.8	6.0–7.3

- **LOSS ON DRYING (731):** Dry 100 mg in a vacuum at a pressure not exceeding 5 mm of mercury at 110° for 3 h; it loses NMT 13.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate whether its molar ratio of amikacin to H<sub>2</sub>SO<sub>4</sub> is 1:2 or 1:1.8.
- **USP REFERENCE STANDARDS (11)**
  - USP Amikacin RS
  - USP Amikacin Sulfate RS
  - USP Kanamycin Sulfate RS

**Amikacin Sulfate Injection****DEFINITION**

Amikacin Sulfate Injection is a sterile solution of Amikacin Sulfate in Water for Injection, or of Amikacin in Water for Injection prepared with the aid of Sulfuric Acid. It contains NLT 90.0% and NMT 120.0% of the labeled amount of amikacin (C<sub>22</sub>H<sub>43</sub>N<sub>5</sub>O<sub>13</sub>).

**IDENTIFICATION**

- **A.** The retention time of the peak for amikacin of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** 0.115 N sodium hydroxide  
**System suitability solution:** 0.02 mg/mL of USP Amikacin RS and 0.008 mg/mL of USP Kanamycin Sulfate RS in water  
**Standard solution:** 0.02 mg/mL of USP Amikacin RS in water  
**Sample solution:** 0.02 mg/mL of amikacin, from the Injection in water  
**Chromatographic system**  
 (See Chromatography (621), *System Suitability*.)  
**Mode:** LC  
**Detector:** Electrochemical  
**Detector mode:** Integrated amperometric mode  
**Electrodes**  
**Working:** Gold  
**Reference:** Silver–silver chloride  
**Detector settings:** See Table 1.

Table 1

No.	Potential		Time (ms)
		V	
E <sub>1</sub>		0.04	200
E <sub>2</sub>		0.8	190
E <sub>3</sub>		−0.8	190

**Columns**

**Guard:** Packing L47  
**Analytical:** 4-mm × 25-cm; 10-μm packing L47  
**Flow rate:** 0.5 mL/min  
**Injection size:** 20 μL  
**System suitability**  
**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for kanamycin and amikacin are 0.8 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between kanamycin and amikacin, *System suitability solution*

**Tailing factor:** NMT 2, *Standard solution*

**Relative standard deviation:** NMT 3%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amikacin (C<sub>22</sub>H<sub>43</sub>N<sub>5</sub>O<sub>13</sub>) in each mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak area from the *Sample solution*  
 $r_S$  = peak area from the *Standard solution*  
 $C_S$  = concentration of USP Amikacin RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of amikacin in the *Sample solution*  
 $P$  = potency of amikacin in USP Amikacin RS (mg/mg)

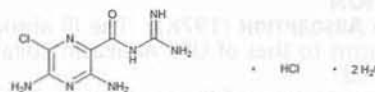
**Acceptance criteria:** 90.0%–120.0%

**SPECIFIC TESTS**

- **PH (791):** 3.5–5.5
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.33 USP Endotoxin Unit/mg of amikacin
- **OTHER REQUIREMENTS:** Meets the requirements under *Injections and Implanted Drug Products (1)*

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type III glass.
- **USP REFERENCE STANDARDS (11)**
  - USP Amikacin RS
  - USP Endotoxin RS
  - USP Kanamycin Sulfate RS

**Amiloride Hydrochloride**

C<sub>6</sub>H<sub>8</sub>ClN<sub>7</sub>O · HCl · 2H<sub>2</sub>O 302.12  
 Pyrazinecarboxamide, 3,5-diamino-N-(aminoiminomethyl)-6-chloro-, monohydrochloride dihydrate;  
 N-Amidino-3,5-diamino-6-chloropyrazinecarboxamide monohydrochloride dihydrate [17440-83-4].

**DEFINITION**

Amiloride Hydrochloride contains NLT 98.0% and NMT 101.0% of C<sub>6</sub>H<sub>8</sub>ClN<sub>7</sub>O · HCl, calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197M)**
- **B. ULTRAVIOLET ABSORPTION (197U)**  
**Sample:** 600 μg/mL of water, diluted quantitatively and stepwise with 0.1 N hydrochloric acid to a concentration of about 9.6 μg/mL  
**Acceptance criteria:** Meets the requirements
- **C. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements



**ASSAY****• PROCEDURE**

**Sample:** 450 mg

**Analysis:** Dissolve the *Sample* in 100 mL of glacial acetic acid, add 10 mL of mercuric acetate TS and 15 mL of dioxane. Add crystal violet TS. Titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 26.61 mg of  $C_6H_8ClN_7O \cdot HCl$ .

**Acceptance criteria:** 98.0%–101.0% on the dried basis

**IMPURITIES**

- RESIDUE ON IGNITION (281):** NMT 0.1%

**Delete the following:**

- HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-Jan-2018)

**• ORGANIC IMPURITIES**

**Standard solutions:** Prepare a series of solutions, A, B, C, D, E, and F, of USP Amiloride Hydrochloride RS in a mixture of methanol and chloroform (4:1) having concentrations of 4000, 40, 20, 8, 4, and 2 µg/mL, respectively.

**Sample solution:** 4 mg/mL of Amiloride Hydrochloride in methanol and chloroform (4:1)

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel previously washed with methanol

**Application volume:** 5 µL

**Developing solvent system:** Tetrahydrofuran and 3 N ammonium hydroxide (15:2)

**Analysis**

**Samples:** *Standard solutions* A, B, C, D, E, and F and *Sample solution*

Proceed as directed in the chapter. Dry the spots with a stream of nitrogen, and develop the chromatograms in the solvent system, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow to air-dry, and examine the plate under long-wavelength UV light: the  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of *Standard solution* A. Estimate the levels of any additional spots observed in the chromatogram of the *Sample solution* by comparison with the principal spots in the chromatograms of *Standard solutions* B, C, D, E, and F.

**Acceptance criteria:** The sum of the intensities of any additional spots observed is NMT that of the principal spot obtained from *Standard solution* B (equivalent to 1%).

**SPECIFIC TESTS****• ACIDITY**

**Sample:** 1.0 g

**Analysis:** Dissolve the *Sample* in 100 mL of a mixture of methanol and water (1:1). Titrate with 0.10 N sodium hydroxide to a potentiometric endpoint.

**Acceptance criteria:** NMT 0.30 mL is required (0.1% as HCl).

**• LOSS ON DRYING**

(See *Thermal Analysis* (891).)

[NOTE—The quantity taken for the determination may be adjusted, if necessary, for instrument sensitivity.]

**Sample:** 10 mg

**Analysis:** Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument using the *Sample*. Heat the specimen at the rate of 10°/min between ambient tem-

perature and 225° in an atmosphere of nitrogen at a flow rate of 40 mL/min. From the thermogram determine the accumulated loss in weight between ambient temperature and about 200° on the plateau.

**Acceptance criteria:** 11.0%–13.0%

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers.

- USP REFERENCE STANDARDS (11)**  
USP Amiloride Hydrochloride RS

**Amiloride Hydrochloride Tablets****DEFINITION**

Amiloride Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- B.**

**Standard solution:** 0.2 mg/mL of USP Amiloride Hydrochloride RS in methanol

**Sample solution:** Prepare from finely ground Tablets, equivalent to 0.2 mg/mL of amiloride hydrochloride in methanol. Filter the solution.

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Developing solvent system:** Tetrahydrofuran and 3 N ammonium hydroxide (88:12)

**Application volume:** 10 µL

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Develop the plate until the solvent is about three-fourths of the length of the plate from the origin. Remove the plate from the developing chamber, air-dry, and examine under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

**ASSAY****• PROCEDURE**

**Buffer:** Dissolve 136 g of monobasic potassium phosphate in 800 mL of water. Adjust with phosphoric acid to a pH of 3.0. Dilute with water to 1000 mL.

**Mobile phase:** Methanol, water, and *Buffer* (25:71:4)

**Standard stock solution:** 1.0 mg/mL of USP Amiloride Hydrochloride RS in methanol

**Standard solution:** 0.1 mg/mL of USP Amiloride Hydrochloride RS from *Standard stock solution*, prepared as follows. Transfer 5.0 mL of the *Standard stock solution* to a 50-mL volumetric flask. Add 10.0 mL of methanol, 2.0 mL of 0.1 N hydrochloric acid, and dilute with water to volume.

**Sample solution:** Transfer an amount equivalent to 5 mg of amiloride hydrochloride, from NLT 20 finely powdered Tablets, to a 50-mL volumetric flask containing 15.0 mL of methanol and 2.0 mL of 0.1 N hydrochloric acid. Sonicate for 10 min, dilute with water to volume, sonicate for an additional 10 min, and filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 286 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of amiloride from the *Sample solution*

$r_S$  = peak response of amiloride from the *Standard solution*

$C_S$  = concentration of USP Amiloride Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amiloride hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Instrumental conditions

Mode: UV

Analytical wavelength: 363 nm

Standard solution: USP Amiloride Hydrochloride RS of known concentration in *Medium*. [NOTE—An amount of methanol not to exceed 2% of the total volume of the *Standard solution* may be used to dissolve the amiloride hydrochloride.]

Sample solution: Sample per *Dissolution* (711). Dilute with *Medium* as necessary.

Analysis

Samples: *Standard solution* and *Sample solution*

Tolerances: NLT 80% (Q) of the labeled amount of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905)

Procedure for content uniformity

Standard solution: 10 µg/mL of USP Amiloride Hydrochloride RS in 0.1 N hydrochloric acid

Sample solution: 10 µg/mL of amiloride hydrochloride prepared as follows. Transfer one finely powdered Tablet to a 100-mL volumetric flask, add 60 mL of 0.1 N hydrochloric acid, and shake by mechanical means for 30 min. Dilute with 0.1 N hydrochloric acid to volume, and centrifuge a portion of the mixture. Dilute a portion of the clear supernatant to obtain the required concentration.

Instrumental conditions

Mode: UV

Analytical wavelength: 363 nm

Blank: 0.1 N hydrochloric acid

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of amiloride hydrochloride in the *Sample solution*

$A_S$  = absorbance of amiloride hydrochloride in the *Standard solution*

$C_S$  = concentration of USP Amiloride Hydrochloride RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of amiloride hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

Diluent: Methanol, 1 N hydrochloric acid, and water (40:4:56)

Buffer: 0.9 g/L of sodium 1-hexanesulfonate in water. Initially add water to about 90% of the volume of the flask, adjust with diluted phosphoric acid to a pH of 3.0 ± 0.1, and dilute with water to volume.

Mobile phase: Acetonitrile and *Buffer* (10:90)

Standard solution: 0.01 mg/mL of USP Amiloride Hydrochloride RS in *Diluent*

Sample solution: Nominally equivalent to 2 mg/mL of amiloride hydrochloride in *Diluent* from NLT 20 powdered Tablets. Initially add methanol to fill about 40% of the volume of the flask and 1 N hydrochloric acid to about 4% of the volume of the flask. Sonicate for 10 min, dilute with water to volume, and sonicate for another 10 min. Pass through a suitable filter of 0.45-µm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 350 nm

Column: 4.6-mm × 15-cm; 4-µm packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 3.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of amiloride from the *Standard solution*

$C_S$  = concentration of USP Amiloride Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amiloride hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chloropyrazine acid <sup>a,e</sup>	0.15	—
Chloropyrazine methyl carboxylate <sup>b,e</sup>	0.48	—
Chloropyrazine carboxamide <sup>c,e</sup>	0.56	—
Amiloride	1.00	—

<sup>a</sup> 3,5,-Diamin-6-chloropyrazine carboxylic acid.

<sup>b</sup> 3,5,-Diamin-6-chloropyrazine-2-carboxylate.

<sup>c</sup> N-Amidino-3-amino-5-hydroxy-6-chloropyrazine-2-carboxamide hydrochloride.

<sup>d</sup> Total impurities is the sum of all the impurities including process-related. Disregard peaks less than 0.05%.

<sup>e</sup> Process-related impurity, controlled in the drug substance.



Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any other unknown impurity	—	0.5
Total impurities <sup>d</sup>	—	2.0

<sup>a</sup> 3,5,-Diamin-6-chloropyrazine carboxylic acid.

<sup>b</sup> 3,5,-Diamin-6-chloropyrazine-2-carboxylate.

<sup>c</sup> N-Ammidine-3-amino-5-hydroxy-6-chloropyrazine-2-carboxamide hydrochloride.

<sup>d</sup> Total impurities is the sum of all the impurities including process-related. Disregard peaks less than 0.05%.

<sup>e</sup> Process-related impurity, controlled in the drug substance.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
USP Amiloride Hydrochloride RS

## Amiloride Hydrochloride and Hydrochlorothiazide Tablets

### DEFINITION

Amiloride Hydrochloride and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) and hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ).

### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.
- **B. THIN-LAYER CHROMATOGRAPHY**  
**Standard solution A:** 0.2 mg/mL of USP Amiloride Hydrochloride RS in methanol  
**Standard solution B:** 2 mg/mL of USP Hydrochlorothiazide RS in methanol  
**Sample solution:** Equivalent to 0.2 mg/mL of amiloride hydrochloride from ground Tablets in methanol. Filter.  
**Chromatographic system**  
 (See Chromatography (621), Thin-Layer Chromatography.)  
**Mode:** TLC  
**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture  
**Application volume:** 10  $\mu$ L  
**Developing solvent system:** Tetrahydrofuran and 3 N ammonium hydroxide (22:3)  
**Analysis**  
**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*  
 Develop the chromatogram until the solvent has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, air-dry, and examine under short-wavelength UV light.  
**Acceptance criteria:** The  $R_f$  values of the amiloride hydrochloride and hydrochlorothiazide spots of the *Sample solution* correspond to those of the corresponding *Standard solutions*.

### ASSAY

#### PROCEDURE

**Buffer:** 136 g of monobasic potassium phosphate in 800 mL of water. Adjust with phosphoric acid to a pH of 3.0. Dilute with water to 1000 mL.  
**Mobile phase:** Methanol, water, and *Buffer* (25:71:4)  
**Standard stock solution:** 1.0 mg/mL of USP Amiloride Hydrochloride RS in methanol

**Standard solution:** 0.1 mg/mL of USP Amiloride Hydrochloride RS and 1 mg/mL of USP Hydrochlorothiazide RS, prepared by transferring 10.0 mL of the *Standard stock solution* to a 100-mL volumetric flask containing 100 mg of USP Hydrochlorothiazide RS and 20.0 mL of methanol. Add 4.0 mL of 1 N hydrochloric acid, and dilute with water to volume.

**Sample solution:** Transfer an equivalent to 5 mg of amiloride hydrochloride from powdered Tablets (NLT 20) to a 50-mL volumetric flask. Add 15.0 mL of methanol and 2.0 mL of 1 N hydrochloric acid. Sonicate for 10 min, dilute with water to volume, sonicate for an additional 10 min, and filter.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 286 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for hydrochlorothiazide and amiloride hydrochloride are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between hydrochlorothiazide and amiloride hydrochloride

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of amiloride hydrochloride from the *Sample solution*

$r_S$  = peak response of amiloride hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Amiloride Hydrochloride RS, corrected for loss in weight in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amiloride hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of hydrochlorothiazide from the *Sample solution*

$r_S$  = peak response of hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amounts of  $C_6H_8ClN_7O \cdot HCl$  and  $C_7H_8ClN_3O_4S_2$

### PERFORMANCE TESTS

#### DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

Determine the amount of amiloride hydrochloride and hydrochlorothiazide dissolved using *Analytical procedure 1* or *procedure 2*.

#### Analytical procedure 1

**Amiloride standard solution:** 60 mg of USP Amiloride Hydrochloride RS (equivalent to 52 mg of anhydrous



amiloride hydrochloride) in a 200-mL volumetric flask. Dissolve in and dilute with methanol to volume. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume.

**Hydrochlorothiazide standard solution:** Transfer 100 mg of USP Hydrochlorothiazide RS to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Medium* to volume. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, and dilute with *Medium* to volume.

**Sample solution A:** Pass a portion of the solution under test through a glass fiber filter of 0.45- $\mu$ m pore size.

**Sample solution B:** Transfer 5.0 mL of *Sample solution A* to a 25-mL volumetric flask, and dilute with *Medium* to volume.

**Detector:** UV 363 nm for amiloride hydrochloride, 270 nm for hydrochlorothiazide

**Blank:** *Medium*

#### Analysis

**Samples:** *Amiloride standard solution*, *Hydrochlorothiazide standard solution*, *Sample solution A*, and *Sample solution B*

Calculate the percentage of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) dissolved:

$$\text{Result} = [(A_U \times C_S \times V)/(A_S \times L)] \times 100$$

$A_U$  = absorbance of *Sample solution A*

$C_S$  = concentration of the *Amiloride standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$A_S$  = absorbance of the *Amiloride standard solution*

$L$  = label claim of amiloride (mg/Tablet)

Calculate the correction for the interference of amiloride:

$$A_{UC} = A_{U270} - [(A_{U363} \times F)/5]$$

$A_{UC}$  = corrected absorbance of *Sample solution A*, 270 nm

$A_{U270}$  = absorbance of *Sample solution B*, 270 nm

$A_{U363}$  = absorbance of *Sample solution A*, 363 nm

$$F = A_S \text{ at } 270 \text{ nm} / A_S \text{ at } 363 \text{ nm}$$

$A_S$  = absorbance of the *Amiloride standard solution*

Calculate the percentage of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) dissolved:

$$\text{Result} = [(A_{UC} \times C_S \times V \times D)/(A_S \times L)] \times 100$$

$A_{UC}$  = corrected absorbance of *Sample solution A*, 270 nm

$C_S$  = concentration of the *Hydrochlorothiazide standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$D$  = dilution factor of *Sample solution B*, 25/5

$A_S$  = absorbance of the *Hydrochlorothiazide standard solution*

$L$  = label claim of hydrochlorothiazide (mg/Tablet)

#### Analytical procedure 2

**Buffer and Mobile phase:** Prepare as directed in the *Assay*.

**Standard stock solution:** Use the *Standard solution* from the *Assay*.

**Standard solution:** 5  $\mu$ g/mL of amiloride hydrochloride and 50  $\mu$ g/mL of hydrochlorothiazide from the *Standard stock solution*, in *Medium*

**Sample solution:** Pass a portion of the solution under test through a filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 286 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0% between hydrochlorothiazide and amiloride

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the amount of amiloride hydrochloride ( $C_6H_8ClN_7O \cdot HCl$ ) and hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response of amiloride or hydrochlorothiazide from the *Sample solution*

$r_S$  = peak response of amiloride or hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of amiloride hydrochloride or hydrochlorothiazide in the *Standard solution* (mg/mL)

$L$  = label claim of amiloride hydrochloride or hydrochlorothiazide (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_6H_8ClN_7O \cdot HCl$  and NLT 75% (Q) of the labeled amount of  $C_7H_8ClN_3O_4S_2$  are dissolved.

- **UNIFORMITY OF DOSAGE UNITS, Content Uniformity (905):** Meet the requirements with respect to amiloride hydrochloride and hydrochlorothiazide

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Buffer, Mobile phase, and Sample solution:** Prepare as directed in the *Assay*.

**Standard solution:** 10  $\mu$ g/mL of USP Benzothiadiazine Related Compound A RS in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 286 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for hydrochlorothiazide and amiloride hydrochloride are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between hydrochlorothiazide and amiloride hydrochloride

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

$r_U$  = peak response of benzothiadiazine related compound A from the *Sample solution*

$r_S$  = peak response of benzothiadiazine related compound A from the *Standard solution*

$C_S$  = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of benzothiadiazine in the *Sample solution* (mg/mL)

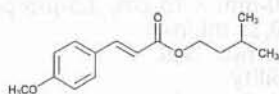


$F$  = unit conversion factor, 0.001 mg/μg  
Acceptance criteria: NMT 1.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**
  - USP Amiloride Hydrochloride RS
  - USP Benzothiadiazine Related Compound A RS
  - 4-Amino-6-chloro-1,3-benzenedisulfonamide.
  - $C_6H_8ClN_3O_4S_2$  285.73
  - USP Hydrochlorothiazide RS

## Amiloxate



$C_{15}H_{20}O_3$  248.32  
4-Methoxycinnamic acid, isoamyl ester;  
3-Methylbutyl 3-(4-methoxyphenyl)-(E)-2-propenoate  
[71617-10-2].

### DEFINITION

Amiloxate contains NLT 98.0% and NMT 102.0% of amiloxate ( $C_{15}H_{20}O_3$ ).

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197F)**

**Change to read:**

- **B. ULTRAVIOLET ABSORPTION (197U)**  
Sample solution: 5.0 μg/mL in alcohol  
▲<sub>USP40</sub>

**Add the following:**

- ▲ **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲<sub>USP40</sub>

### ASSAY

**Change to read:**

- **PROCEDURE**  
Standard solution: 20 mg/mL of USP Amiloxate RS in *tert*-butyl methyl ether  
Sample solution: 20 mg/mL of Amiloxate in *tert*-butyl methyl ether  
**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)  
Mode: GC  
Detector: Flame ionization  
Column: 0.32-mm × 25-m; coated with a 0.1-μm film of phase G1  
Temperatures  
Injection port: 240°  
Detector: 260°  
Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
60	8	240	10

Carrier gas: Helium

Flow rate: 6 mL/min

Injection volume: 1 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 0.73% ▲<sub>USP40</sub>

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amiloxate ( $C_{15}H_{20}O_3$ ) in the portion of Amiloxate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amiloxate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amiloxate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

### IMPURITIES

- **ORGANIC IMPURITIES**

Standard solution, *Sample solution*, *Chromatographic system*, and *System suitability*: Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Amiloxate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_T$  = sum of all the peak responses, excluding the solvent peak, from the *Sample solution*

Acceptance criteria

Individual impurities: NMT 0.5%

Total impurities: NMT 2.0%

### SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 1.037–1.041
- **REFRACTIVE INDEX (831):** 1.556–1.560 at 20°

**Change to read:**

- **ACIDITY**

Sample solution: Transfer 50 mL of alcohol to a suitable container. Add 1 mL of phenolphthalein TS and sufficient 0.1 N sodium hydroxide to obtain a persistent pink color. Transfer 50 mL of this solution to a suitable container, and add 5.0 mL of Amiloxate.

Analysis: Titrate with 0.1 N sodium hydroxide

▲<sub>VS</sub> ▲<sub>USP40</sub>

Acceptance criteria: NMT 0.2 mL of titrant per mL of Amiloxate is required for neutralization.

### ADDITIONAL REQUIREMENTS

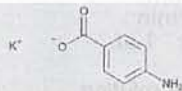
- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
USP Amiloxate RS



## Aminobenzoate Potassium

Add the following:

▲



$C_7H_6KNO_2$  175.23  
Benzoic acid, 4-amino-, potassium salt;  
Potassium 4-aminobenzoate [138-84-1].▲USP40

### DEFINITION

Change to read:

Aminobenzoate Potassium contains NLT ▲98.0%▲USP40 and NMT ▲102.0%▲USP40 of aminobenzoate potassium ( $C_7H_6KNO_2$ ), calculated on the dried basis.

### IDENTIFICATION

Delete the following:

#### ▲ A. ULTRAVIOLET ABSORPTION (197U)

Solution: 10 µg/mL in 0.001 N sodium hydroxide  
Acceptance criteria: Meets the requirements.▲USP40

Add the following:

#### ▲ A. INFRARED ABSORPTION (197K)▲USP40

Delete the following:

#### ▲ B.

Sample: 400 mg

Analysis: Dissolve the Sample in 10 mL of water, add 1 mL of 3 N hydrochloric acid, filter, and wash the precipitate with two 5-mL portions of cold water. Recrystallize the precipitate from alcohol, and dry at 110° for 1 h.

Acceptance criteria: The resulting *p*-aminobenzoic acid melts between 186° and 189°.▲USP40

Add the following:

▲ B. The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.▲USP40

#### • C.

Sample solution: 10 mg/mL of Aminobenzoate Potassium in water

Acceptance criteria: The Sample solution imparts a violet color to a nonluminous flame. Since the presence of small quantities of sodium masks the color, screen out the yellow color produced by sodium by viewing through a blue filter that blocks emission at 589 nm (sodium) but is transparent to emission at 404 nm (potassium). [NOTE—Traditionally, cobalt glass has been used, but other suitable filters are commercially available.]

### ASSAY

Change to read:

#### • PROCEDURE

▲Solution A: 1.5% acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid and passing through a suitable filter of 0.45-µm pore size

Mobile phase: Methanol and Solution A (15:85)

Standard solution: 0.1 mg/mL of USP Aminobenzoate Potassium RS in Mobile phase. Sonicate to dissolve.

Sample solution: 0.1 mg/mL of Aminobenzoate Potassium in Mobile phase. Sonicate to dissolve.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 280 nm

Column: 3.0-mm × 15-cm; 3.5-µm packing L11

Flow rate: 0.35 mL/min

Injection volume: 5 µL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 0.73%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of aminobenzoate potassium ( $C_7H_6KNO_2$ ) in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Aminobenzoate Potassium RS in the Standard solution (mg/mL)

$C_U$  = concentration of Aminobenzoate Potassium in the Sample solution (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis▲USP40

### IMPURITIES

Delete the following:

#### ▲ CHLORIDE AND SULFATE (221), Chloride

Sample: 1.4 g

Acceptance criteria: Shows no more chloride than corresponds to 0.4 mL of 0.020 N hydrochloric acid (0.02%).▲USP40

Delete the following:

#### ▲ CHLORIDE AND SULFATE (221), Sulfate

Sample: 1.4 g

Acceptance criteria: Shows no more sulfate than corresponds to 0.3 mL of 0.020 N sulfuric acid (0.02%).▲USP40

Delete the following:

• HEAVY METALS (231), Method II: NMT 0.002%● (Official 1-Jan-2018)

Delete the following:

#### ▲ VOLATILE DIAZOTIZABLE SUBSTANCES

Standard stock solution: 0.1 mg/mL of *p*-toluidine, prepared by dissolving a quantity of *p*-toluidine in 5% of the flask volume of methanol, and diluting with water to volume



**Standard solution:** 1 µg/mL of *p*-toluidine from the *Standard stock solution*

**Sample solution:** Transfer 5.0 g of Aminobenzoate Potassium to a suitable flask, and add a volume of 1.25 N sodium hydroxide that is just sufficient to dissolve the sample and to render the solution just alkaline to phenolphthalein TS. Dilute with water to 50 mL, and steam-distill the solution, collecting 95 mL of the distillate in a 100-mL volumetric flask. Dilute with water to volume.

**Blank:** Water

#### Instrumental conditions

**Mode:** Vis

**Analytical wavelength:** Wavelength of maximum absorbance at about 405 nm

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank solution*. Transfer 20.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to three separate 100-mL beakers, and treat each as follows. Add 5.0 mL of 1 N hydrochloric acid, and cool in an ice bath. Add 2.0 mL of 0.1 M sodium nitrite dropwise, with stirring. Allow to stand for 5 min for the diazotization reaction to be complete, add quickly to 10.0 mL of a cold solution of guaiacol (freshly prepared by dissolving 0.20 g of guaiacol in 100 mL of 1 N sodium hydroxide), mix and allow to stand for 30 min. Concomitantly determine the absorbances of the solutions.

**Acceptance criteria:** The absorbance of the solution from the *Sample solution* does not exceed that of the solution from the *Standard solution*, corresponding to NMT 0.002% of volatile diazotizable substances as *p*-toluidine. ▲USP40

#### Add the following:

##### ▲• LIMIT OF ANILINE AND *p*-TOLUIDINE

**Diluent:** Methylene chloride

**Standard stock solution:** 0.1 mg/mL each of USP Aniline RS and USP *p*-Toluidine RS in *Diluent*

**Standard solution:** 1 µg/mL each of USP Aniline RS and USP *p*-Toluidine RS in *Diluent* from *Standard stock solution*

**Sample solution:** 100 mg/mL of Aminobenzoate Potassium in *Diluent* prepared as follows. Add an appropriate quantity of Aminobenzoate Potassium to a suitable volumetric flask and dilute with *Diluent* to volume. Agitate for 10 min on a shaker and centrifuge at 3000 rpm for 5 min. Use the supernatant.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

#### Detectors

**Flame ionization:** 300°

**Hydrogen:** 40 mL/min

**Air:** 400 mL/min

**Column:** 30-m × 0.32-mm fused silica capillary; coated with 0.5-µm film of phase G27

#### Temperatures

**Injection port:** 280°

**Detector:** 300°

**Column:** See *Table 1*.

**Table 1**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	—	130	4
130	20	180	5

**Carrier gas:** Helium

**Flow rate:** 1.0 mL/min

**Injection volume:** 2 µL

**Injection type:** Split ratio, 1:10

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times of aniline and *p*-toluidine are about 4.1 and 5.1 min, respectively.]

#### Suitability requirements

**Resolution:** NLT 7.0 between aniline and *p*-toluidine

**Tailing factor:** NMT 1.5 for aniline and *p*-toluidine

**Relative standard deviation:** NMT 6.0% for aniline and *p*-toluidine

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of *p*-toluidine or aniline in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of *p*-toluidine or aniline from the *Sample solution*

$r_S$  = peak response of *p*-toluidine or aniline from the *Standard solution*

$C_S$  = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aminobenzoate Potassium in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Aniline:** NMT 10 ppm

***p*-Toluidine:** NMT 20 ppm ▲USP40

#### Add the following:

##### ▲• ORGANIC IMPURITIES

**Solution A:** 1.5% acetic acid, prepared by mixing 690 mL of water with 10 mL of glacial acetic acid

**Solution B:** Methanol

**Mobile phase:** See *Table 2*.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0.0	85	15
4.0	85	15
4.1	45	55
10.0	45	55
10.1	85	15
13	85	15

**Diluent:** Methanol and water (85:15)

**System suitability solution:** 1 mg/mL of USP Aminobenzoate Potassium RS, 0.01 mg/mL of USP 4-Nitrobenzoic Acid RS, and 0.01 mg/mL of USP Benzocaine RS in *Diluent* prepared as follows. Transfer 1 mL each of 0.1 mg/mL of USP 4-Nitrobenzoic Acid RS in methanol and 0.1 mg/mL of USP Benzocaine RS in *Diluent* to a 10-mL volumetric flask containing the appropriate amount of USP Aminobenzoate Potassium RS, and dilute with *Diluent* to volume.

**Standard solution:** 1 µg/mL each of USP Aminobenzoate Potassium RS, USP 4-Nitrobenzoic Acid RS, and USP Benzocaine RS in *Diluent*

**Sample solution:** 1 mg/mL of Aminobenzoate Potassium in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 280 nm

Column: 3.0-mm × 15-cm; 3.5-μm packing L11

Flow rate: 0.4 mL/min

Injection volume: 5 μL

#### System suitability

Samples: System suitability solution and Standard solution

#### Suitability requirements

Resolution: NLT 1.5 between benzocaine and 4-nitrobenzoic acid, System suitability solution

Relative standard deviation: NMT 3% for the aminobenzoate potassium, 4-nitrobenzoic acid, and benzocaine peaks, Standard solution

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of 4-nitrobenzoic acid or benzocaine in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of 4-nitrobenzoic acid or benzocaine from the Sample solution

$r_S$  = peak response of 4-nitrobenzoic acid or benzocaine from the Standard solution

$C_S$  = concentration of USP 4-Nitrobenzoic Acid RS or USP Benzocaine RS in the Standard solution (mg/mL)

$C_U$  = concentration of Aminobenzoate Potassium in the Sample solution (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Aminobenzoate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity from the Sample solution

$r_S$  = peak response of aminobenzoate from the Standard solution

$C_S$  = concentration of USP Aminobenzoate Potassium RS in the Standard solution (mg/mL)

$C_U$  = concentration of Aminobenzoate Potassium in the Sample solution (mg/mL)

Acceptance criteria: See Table 3.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—
Benzocaine	2.0	0.2
4-Nitrobenzoic acid	2.1	0.2
Any individual unspecified impurity	—	0.10

▲USP40

#### SPECIFIC TESTS

##### • PH (791)

Sample solution: 50 mg/mL of Aminobenzoate Potassium in water

Acceptance criteria: 8.0–9.0

##### • LOSS ON DRYING (731)

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 1.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

#### Change to read:

##### • USP REFERENCE STANDARDS (11)

USP Aminobenzoate Potassium RS

▲USP Aniline RS

Aniline,

$C_6H_7N$  93.13

USP Benzocaine RS

USP 4-Nitrobenzoic Acid RS

4-Nitrobenzoic acid,

$C_7H_5NO_4$  167.12

USP *p*-Toluidine RS

4-Methylaniline,

$C_7H_9N$  107.15▲USP40

## Aminobenzoate Potassium Capsules

#### DEFINITION

Aminobenzoate Potassium Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of aminobenzoate potassium ( $C_7H_6KNO_2$ ).

#### IDENTIFICATION

##### • A.

Sample: 1 g of the Capsule contents

Analysis: Dissolve the Sample in 25 mL of water, add 5 mL of 3 N hydrochloric acid, and wash the precipitate with two 5-mL portions of cold water. Recrystallize from alcohol the precipitate so obtained, and dry at 110° for 1 h.

Acceptance criteria: The *p*-aminobenzoic acid melts between 186° and 189°.

#### Add the following:

▲• **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.▲USP40

#### ASSAY

#### Change to read:

##### • PROCEDURE

▲Solution A: 1.5% acetic acid prepared as follows. Mix 690 mL of water with 10 mL of acetic acid and pass through a filter of 0.45-μm pore size.

Mobile phase: Methanol and Solution A (15:85)

Standard solution: 0.1 mg/mL of USP Aminobenzoate Potassium RS in Mobile phase

Sample solution: Nominally 0.1 mg/mL of aminobenzoate potassium prepared as follows. Remove as completely as possible, and combine the contents of NLT 10 Capsules. Transfer a portion of the combined contents, equivalent to 10 mg of aminobenzoate potassium to a 100-mL volumetric flask, dissolve in 70 mL of Mobile phase, sonicate for 3–4 min, and dilute with Mobile phase to volume.

#### Chromatographic system

(See Chromatography (621), System Suitability.)



Mode: LC  
 Detector: 280 nm  
 Column: 3.0-mm × 15-cm; 3.5-μm packing L11  
 Flow rate: 0.35 mL/min  
 Injection volume: 5 μL

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aminobenzoate potassium ( $C_7H_6KNO_2$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aminobenzoate potassium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110%  $\Delta$ USP40

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

#### Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 270 nm

Standard solution: A known concentration of USP Aminobenzoate Potassium RS in *Medium*

Sample solution: Filter portions of the solution under test, and dilute with *Medium*, if necessary, in comparison with the *Standard solution* concentration.

Analysis: Calculate the percentage of the labeled amount of aminobenzoate potassium ( $C_7H_6KNO_2$ ) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of aminobenzoate potassium ( $C_7H_6KNO_2$ ) is dissolved.

##### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

#### IMPURITIES

##### Add the following:

##### ▲• ORGANIC IMPURITIES

Solution A, *Mobile phase*, and *Chromatographic system*: Prepare as directed in the *Assay*.

Standard solution: 1 μg/mL each of USP Aminobenzoate Potassium RS, USP 4-Nitrobenzoic Acid RS, and USP Benzocaine RS in *Mobile phase*

Sensitivity solution: 0.1 μg/mL of USP Aminobenzoate Potassium RS in *Mobile phase* from the *Standard solution*

Sample solution: Nominally 1 mg/mL of aminobenzoate potassium in *Mobile phase* prepared as follows. Remove as completely as possible, and combine, the contents of NLT 10 Capsules. Transfer a portion of the combined contents, equivalent to 10 mg of aminobenzoate potassium, to a 10-mL volumetric flask. Dissolve in 7 mL of *Mobile phase*, sonicate for 3–4 min, and dilute with *Mobile phase* to volume.

#### System suitability

Samples: *Standard solution* and *Sensitivity solution*

#### Suitability requirements

Resolution: NLT 1.5 between benzocaine and 4-nitrobenzoic acid, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any individual unspecified degradation product from the *Sample solution*

$r_S$  = peak response of aminobenzoate from the *Standard solution*

$C_S$  = concentration of USP Aminobenzoate Potassium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aminobenzoate potassium in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—
Benzocaine <sup>a</sup>	2.0	—
4-Nitrobenzoic acid <sup>a</sup>	2.1	—
Any individual unspecified degradation product	—	0.10
Total impurities	—	1.0

<sup>a</sup>These are process impurities controlled in the API and are included in this table for identification purposes only. They are not reported in the drug product and should not be included in the total impurities.

$\Delta$ USP40

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

#### Change to read:

##### • USP REFERENCE STANDARDS (11)

USP Aminobenzoate Potassium RS

$\Delta$ USP Benzocaine RS

USP 4-Nitrobenzoic Acid RS

4-Nitrobenzoic acid.

$C_7H_5NO_4$  167.12  $\Delta$ USP40

### Aminobenzoate Potassium for Oral Solution

» Aminobenzoate Potassium for Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aminobenzoate potassium ( $C_7H_6KNO_2$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Aminobenzoate Potassium RS



**Identification—****A: Ultraviolet Absorption** (197U)—**Solution:** 50 µg per mL.**Medium:** water.

**B:** Dissolve about 400 mg in 10 mL of water, add 1 mL of 3 N hydrochloric acid, filter, and wash the precipitate with two 5-mL portions of cold water. Recrystallize from alcohol the precipitate so obtained, and dry at 110° for 1 hour: the *p*-aminobenzoic acid so obtained melts between 186° and 189°.

**Minimum fill** (755)—

FOR SOLID PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 7.0 and 9.0, in a solution (1 in 10).

**Assay**—Transfer about 100 mg of Aminobenzoate Potassium for Oral Solution, accurately weighed, to a suitable vessel, add 5 mL of hydrochloric acid and 50 mL of water, mix, cool to 15°, and add 25 g of crushed ice. Titrate with 0.1 M sodium nitrite VS, determining the endpoint potentiometrically, using a calomel-platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 17.52 mg of aminobenzoate potassium ( $C_7H_6KNO_2$ ).

## Aminobenzoate Potassium Tablets

» Aminobenzoate Potassium Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aminobenzoate potassium ( $C_7H_6KNO_2$ ).

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Aminobenzoate Potassium RS

**Identification**—Proceed as directed for *Aminobenzoate Potassium Capsules*, using 1 g of finely powdered Tablets.

**Dissolution** (711)—**Medium:** water; 900 mL.**Apparatus 1:** 100 rpm.**Time:** 45 minutes.

**Procedure**—Determine the amount of  $C_7H_6KNO_2$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 270 nm on filtered portions of the solution under test, suitably diluted with *Medium*, in comparison with a Standard solution having a known concentration of USP Aminobenzoate Potassium RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_7H_6KNO_2$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Standard preparation**—Prepare a solution of USP Aminobenzoate Potassium RS having a known concentration of about 5 µg per mL.

**Assay preparation and Procedure**—Weigh and finely powder not fewer than 20 Tablets. Using a portion of the powdered Tablets, equivalent to about 100 mg of aminobenzoate potassium, proceed as directed in the *Assay* under *Aminobenzoate Potassium Capsules*.

## Aminobenzoate Sodium

**DEFINITION**

Aminobenzoate Sodium contains NLT 98.5% and NMT 101.0% of aminobenzoate sodium ( $C_7H_6NNaO_2$ ), calculated on the dried basis.

**IDENTIFICATION**• **A. ULTRAVIOLET ABSORPTION** (197U)**Solution:** 10 µg/mL**Medium:** 0.001 N sodium hydroxide**Acceptance criteria:** Meets the requirements• **B.**

**Sample:** Dissolve about 400 mg of Aminobenzoate Sodium in 10 mL of water, add 1 mL of 3 N hydrochloric acid, filter, and wash the precipitate with two 5-mL portions of cold water. Recrystallize the precipitate so obtained from alcohol, and dry at 110° for 1 h.

**Acceptance criteria:** The resulting *p*-aminobenzoic acid melts between 186° and 189°.

• **C.**

**Sample solution:** 10 mg/mL of Aminobenzoate Sodium in water

**Acceptance criteria:** The *Sample solution* imparts an intense yellow color to a nonluminous flame.

**ASSAY**• **PROCEDURE**

**Sample:** 500 mg of Aminobenzoate Sodium

**Titrimetric system****Mode:** Direct titration**Titrant:** 0.1 M sodium nitrite VS**Endpoint detection:** Potentiometric

**Analysis:** Add 25 mL of water and 25 mL of 3 N hydrochloric acid to the *Sample* in a suitable vessel, mix, and cool in an ice bath. Titrate with *Titrant* using a calomel-platinum electrode system. Each mL of 0.1 M sodium nitrite is equivalent to 15.91 mg of aminobenzoate sodium ( $C_7H_6NNaO_2$ ).

**Acceptance criteria:** 98.5%–101.0% on the dried basis

**IMPURITIES**• **CHLORIDE AND SULFATE** (221), *Chloride***Sample:** 1.4 g of Aminobenzoate Sodium

**Acceptance criteria:** The *Sample* shows no more chloride than corresponds to 0.4 mL of 0.020 N hydrochloric acid (0.02%).

• **CHLORIDE AND SULFATE** (221), *Sulfate***Sample:** 1.4 g of Aminobenzoate Sodium

**Acceptance criteria:** The *Sample* shows no more sulfate than corresponds to 0.3 mL of 0.020 N sulfuric acid (0.02%).

**Delete the following:**• **HEAVY METALS** (231), *Method II:* NMT 0.002% (Official 1-

Jan-2018)

• **VOLATILE DIAZOTIZABLE SUBSTANCES**

**Standard stock solution:** 0.1 mg/mL of *p*-toluidine, prepared as follows. Dissolve an adequate amount of *p*-toluidine in 5% of total volume of methanol in a suitable volumetric flask, and dilute with water to volume.

**Standard solution:** 1 µg/mL of *p*-toluidine in water from the *Standard stock solution*

**Sample solution:** Transfer 5.0 g of Aminobenzoate Sodium to a suitable flask, and add a volume of 1.25 N sodium hydroxide that is just sufficient to dissolve the sample and to render the solution just alkaline to phenolphthalein TS. Dilute with water to 50 mL, and steam-distill the solution, collecting 95 mL of the distillate in a 100-mL volumetric flask. Dilute with water to volume.



Blank: Water

Instrumental conditions

Mode: Vis

Analytical wavelength: Maximum absorbance at about 405 nm

Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
Transfer 20.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to three separate 100-mL beakers, and treat each as follows. Add 5.0 mL of 1 N hydrochloric acid, and cool in an ice bath. Add 2.0 mL of 0.1 M sodium nitrite dropwise, with stirring. Allow to stand for 5 min for the diazotization reaction to be complete, add quickly to 10.0 mL of a cold solution of guaiacol (freshly prepared by dissolving 0.20 g of guaiacol in 100 mL of 1 N sodium hydroxide), and allow to stand for 30 min. Concomitantly determine the absorbances of the solutions.

**Acceptance criteria:** The absorbance of the *Sample solution* does not exceed that of the *Standard solution*, corresponding to NMT 0.002% of volatile diazotizable substances as *p*-toluidine.

## SPECIFIC TESTS

### • PH (791)

**Sample solution:** 50 mg/mL of Aminobenzoate Sodium in water

**Acceptance criteria:** 8.0–9.0

### • LOSS ON DRYING (731)

**Analysis:** Dry at 105° for 2 h.

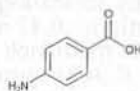
**Acceptance criteria:** NMT 1.0%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**  
USP Aminobenzoate Sodium RS

## Aminobenzoic Acid



$C_7H_7NO_2$  137.14  
Benzoic acid, 4-amino;  
*p*-Aminobenzoic acid [150-13-0].

## DEFINITION

Aminobenzoic Acid contains NLT 98.0% and NMT 102.0% of aminobenzoic acid ( $C_7H_7NO_2$ ), calculated on the dried basis.

## IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### • PROCEDURE

**Acetic acid solution:** Glacial acetic acid and water (1:69)

**Mobile phase:** Methanol and *Acetic acid solution* (15:85)

**Standard solution:** 0.1 mg/mL of USP Aminobenzoic Acid RS in *Mobile phase*. Sonicate to aid dissolution.

**Sample solution:** 0.1 mg/mL of Aminobenzoic Acid in *Mobile phase*. Sonicate to aid dissolution.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.0-mm × 15-cm; 3.5-μm packing L11

**Flow rate:** 0.4 mL/min

**Injection volume:** 5 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing:** NMT 1.5

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid

( $C_7H_7NO_2$ ) in the portion of Aminobenzoic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

## IMPURITIES

• **RESIDUE ON IGNITION (281):** NMT 0.1%

**Delete the following:**

• **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-

Jan-2018)

## • ORGANIC IMPURITIES

**Solution A:** Acetonitrile and methanol (70:80)

**Buffer:** 1.5 g/L of monobasic potassium phosphate and 2.5 g/L of octanesulfonic acid sodium salt in water. Adjust with phosphoric acid to a pH of 2.2.

**Mobile phase:** *Solution A* and *Buffer* (20:80)

**Standard stock solution:** 0.25 mg/mL each of USP

Benzocaine RS and 4-nitrobenzoic acid in methanol

**Standard solution:** 0.5 μg/mL each of USP Benzocaine RS and 4-nitrobenzoic acid in *Mobile phase*, from the *Standard stock solution*

**Sample solution:** 0.25 mg/mL of Aminobenzoic Acid in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.0-mm × 15-cm; 5-μm packing L7

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 μL

**Run time:** 11 times the retention time of the aminobenzoic acid peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 4-nitrobenzoic acid or any unspecified impurity in the portion of Aminobenzoic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of 4-nitrobenzoic acid or any unspecified impurity from the *Sample solution*

$r_S$  = peak response of 4-nitrobenzoic acid from the *Standard solution*

$C_S$  = concentration of 4-nitrobenzoic acid in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aminobenzoic Acid in the *Sample solution* (mg/mL)

Calculate the percentage of benzocaine in the portion of Aminobenzoic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response of benzocaine from the *Sample solution*  
 $r_S$  = peak response of benzocaine from the *Standard solution*  
 $C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Aminobenzoic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1. Disregard any impurity peaks less than 0.02%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	1.0	—
4-Nitrobenzoic acid	4.0	0.2
Benzocaine	9.0	0.2
Any individual unspecified impurity	—	0.1
Total impurities	—	0.5

#### • LIMIT OF ANILINE AND *p*-TOLUIDINE

Diluent: 84 g/L of sodium hydroxide in water

Standard solution: 1.0 µg/mL each of aniline and *p*-toluidine in methylene chloride

Sample solution: Dissolve 1 g of Aminobenzoic Acid in 10.0 mL of Diluent, and extract with two quantities each of 10.0 mL of methylene chloride. Combine, and wash with 5 mL of water. Filter through anhydrous sodium sulfate, and wash the filter with methylene chloride. Evaporate in a water bath at 50°–60° to obtain a volume of about 1–5 mL. Transfer to a 10-mL volumetric flask, and dilute with methylene chloride to volume.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm × 30-m fused silica capillary, coated with a 0.5-µm film of phase G27

Temperatures

Injection port: 280°

Detector: 300°

Column: See Table 2.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	0	130	4
130	20	180	5

Carrier gas: Helium

Flow rate: 1.0 mL/min

Injection volume: 2 µL

Split ratio: 1:10

#### Analysis

Samples: *Standard solution* and *Sample solution*

[NOTE—The relative retention times for aniline and *p*-toluidine are about 0.8 and 1.0, respectively.]

Calculate, in ppm, the amount of aniline and *p*-toluidine in the portion of Aminobenzoic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 10^6$$

- $r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of the corresponding impurity from the *Standard solution*  
 $C_S$  = concentration of the corresponding impurity in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aminobenzoic Acid in the *Sample solution* (mg/mL)

#### Acceptance criteria

Aniline: NMT 10 ppm

*p*-Toluidine: NMT 10 ppm

#### SPECIFIC TESTS

##### • LOSS ON DRYING (731)

Analysis: Dry a sample at 105° for 2 h.

Acceptance criteria: NMT 0.2%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

##### • USP REFERENCE STANDARDS (11)

USP Aminobenzoic Acid RS

USP Benzocaine RS

Benzoic acid, 4-amino-, ethyl ester.

C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub> 165.19

## Aminobenzoic Acid Gel

#### DEFINITION

Aminobenzoic Acid Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of aminobenzoic acid (C<sub>7</sub>H<sub>7</sub>NO<sub>2</sub>).

#### IDENTIFICATION

##### • A. INFRARED ABSORPTION (197K)

##### • B. ULTRAVIOLET ABSORPTION (197U)

Sample solution: 5 µg/mL in alcohol

Acceptance criteria: Meets the requirements

#### ASSAY

##### • PROCEDURE

Mobile phase: Methanol, glacial acetic acid, and water (30:1:69). Allow the mixture to cool, and pass, if necessary, through a suitable microporous membrane filter, and degas.

Internal standard solution: 7 mg/mL of salicylic acid in methanol; dissolve by sonicating.

Standard stock solution: 0.42 mg/mL of USP Aminobenzoic Acid RS in methanol; dissolve by sonicating.

Standard solution: 0.042 mg/mL of USP Aminobenzoic Acid RS in methanol prepared as follows. Transfer 5 mL each of *Standard stock solution* and *Internal standard solution* into a 50-mL volumetric flask, and dilute with methanol to volume. Pass through 0.6-µm filter paper. Throughout the preparation, protect against actinic light.

Sample solution: Nominally 0.042 mg/mL of aminobenzoic acid in methanol prepared as follows. Transfer a quantity of Gel, equivalent to 4.2 mg of aminobenzoic acid, into a 100-mL volumetric flask. Add 10.0 mL of *Internal standard solution* and 50 mL of methanol. Shake or sonicate, as necessary, and dilute with methanol to volume. Filter, if necessary, through filter paper (Whatman No. 41 or equivalent). Pass through 0.6-µm filter paper. Throughout this preparation, protect against actinic light.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 280 nm

Column: 3.9-mm × 30-cm; packing L11

Flow rate: 1.0 mL/min

Injection volume: 15 µL

#### System suitability

Sample: *Standard solution*

Chromatograph replicate 15-µL injections of the *Standard solution* until the response ratio variability is within 1.0% of average.



[NOTE—The relative retention times for aminobenzoic acid and salicylic acid are about 1.0 and 3.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between aminobenzoic acid and salicylic acid peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aminobenzoic acid ( $C_7H_7NO_2$ ) in the portion of Gel taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of aminobenzoic to salicylic acid from the *Sample solution*

$R_S$  = peak response ratio of aminobenzoic to salicylic acid from the *Standard solution*

$C_S$  = concentration of USP Aminobenzoic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aminobenzoic acid in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method II (611):** 42.3%–54.0% (w/w) of  $C_2H_5OH$

#### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

#### SPECIFIC TESTS

- **PH (791):** 4.0–6.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Aminobenzoic Acid RS

### Aminobenzoic Acid Topical Solution

#### DEFINITION

Aminobenzoic Acid Topical Solution contains, in each mL, NLT 45 mg and NMT 55 mg of aminobenzoic acid ( $C_7H_7NO_2$ ).

#### IDENTIFICATION

##### A.

**Sample:** 1 mL of Topical Solution

**Analysis:** To the *Sample* add 1 mL of 1 N sodium hydroxide, and add, in order, 0.5 mL of potassium iodide TS, 0.5 mL of 3 N hydrochloric acid, and 0.5 mL of sodium hypochlorite TS.

**Acceptance criteria:** A brown precipitate is formed.

##### B.

**Sample:** 1 mL of Topical Solution

**Analysis:** To the *Sample* add 2 mL of 3 N hydrochloric acid, and cool to 10°. Add 1 mL of a 10-mg/mL sodium nitrite solution, then add a solution prepared by mixing 50 mg of 2-naphthol with 3 mL of a 100-mg/mL sodium hydroxide solution.

**Acceptance criteria:** A red color is produced.

#### ASSAY

##### PROCEDURE

**Sample:** 5 mL of Topical Solution

**Analysis:** Transfer the *Sample* to a suitable open vessel, evaporate on a steam bath to dryness, and proceed as directed in *Nitrite Titration* (451). Each mL of 0.1 M sodium nitrite is equivalent to 13.71 mg of aminobenzoic acid ( $C_7H_7NO_2$ ).

**Acceptance criteria:** 45–55 mg/mL

#### OTHER COMPONENTS

- **ALCOHOL DETERMINATION (611):** 65%–75%

#### SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 0.895–0.905

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

### Aminocaproic Acid



$C_6H_{13}NO_2$

Hexanoic acid, 6-amino-

6-Aminohexanoic acid [60-32-2].

131.17

#### DEFINITION

Aminocaproic Acid contains NLT 98.5% and NMT 101.5% of aminocaproic acid ( $C_6H_{13}NO_2$ ), calculated on the anhydrous basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

#### ASSAY

##### PROCEDURE

**Solution A:** 0.55 g/L of sodium 1-heptanesulfonate in water

**Mobile phase:** Dissolve 10 g of monobasic potassium phosphate in 300 mL of *Solution A*, and add 250 mL of methanol, followed by another 300 mL of *Solution A*. Adjust the mixture with phosphoric acid to a pH of 2.2, and dilute with *Solution A* to 1 L.

**Internal standard solution:** 1.25 mg/mL of methionine in water

**Standard stock solution:** 12.5 mg/mL of USP Aminocaproic Acid RS in water

**Standard solution:** 5.0 mL of *Standard stock solution* and 2.0 mL of *Internal standard solution* in a 100-mL volumetric flask. Dilute with water to volume.

**Sample stock solution:** 12.5 mg/mL of Aminocaproic Acid in water

**Sample solution:** 5.0 mL of *Sample stock solution* and 2.0 mL of *Internal standard solution* in a 100-mL volumetric flask. Dilute with water to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Column temperature:** 30°

**Flow rate:** 0.7 mL/min

**Run time:** NLT 2 times the retention time of aminocaproic acid

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for aminocaproic acid and methionine are about 0.76 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between aminocaproic acid and methionine



Relative standard deviation: NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of aminocaproic acid ( $C_6H_{13}NO_2$ ) in the portion of Aminocaproic Acid taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of aminocaproic acid to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of aminocaproic acid to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Aminocaproic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aminocaproic Acid in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.5%–101.5% on the anhydrous basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

#### Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm (Official 1-Jan-2018)

#### SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* (921): NMT 0.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Aminocaproic Acid RS

### Aminocaproic Acid Injection

#### DEFINITION

Aminocaproic Acid Injection is a sterile solution of Aminocaproic Acid in Water for Injection. It contains NLT 95.0% and NMT 107.5% of the labeled amount of aminocaproic acid ( $C_6H_{13}NO_2$ ).

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

**Sample:** Mix 2 mL of Injection, added dropwise, with 100 mL of acetone, rapidly stirring the mixture with a glass rod to induce crystallization. Allow the mixture to stand for 15 min, and pass through a sintered-glass filter of medium pore size. Wash the crystals with 25 mL of acetone, apply a vacuum to remove the solvent, dry at 105° for 30 min, and cool. Use the residue.

**Acceptance criteria:** Meets the requirements

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Transfer 11 g of sodium 1-pentanesulfonate and 40 g of anhydrous sodium sulfate to a 2-L volumetric flask, and dissolve in about 500 mL of water. Add 20 mL of 1 N sulfuric acid and 30 mL of acetonitrile, and dilute with water to volume.

**Standard solution:** 2.5 mg/mL of USP Aminocaproic Acid RS in *Mobile phase*

**System suitability solution:** Mix 20 µL of benzyl alcohol with 100 mL of water. Dilute 1.0 mL of this solution with *Standard solution* to 10 mL.

**Sample stock solution:** Nominally equivalent to 25 mg/mL of aminocaproic acid from a suitable volume of Injection in water

**Sample solution:** 2.5 mg/mL of *Sample stock solution* in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 50 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 7.0 between benzyl alcohol and aminocaproic acid, *System suitability solution*

[NOTE—The aminocaproic acid peak elutes before the benzyl alcohol peak.]

**Relative standard deviation:** NMT 1.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminocaproic acid ( $C_6H_{13}NO_2$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Aminocaproic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aminocaproic acid in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–107.5%

#### SPECIFIC TESTS

- **PH** (791): 6.0–7.6
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.05 USP Endotoxin Unit/mg of aminocaproic acid
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS** (11)  
USP Aminocaproic Acid RS  
USP Endotoxin RS

### Aminocaproic Acid Oral Solution

#### DEFINITION

Aminocaproic Acid Oral Solution contains NLT 95.0% and NMT 115.0% of the labeled amount of aminocaproic acid ( $C_6H_{13}NO_2$ ).

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

**Sample:** Mix 1 g of ion-exchange resin (strongly acidic styrene-divinylbenzene cation-exchange resin) with 10 mL of 1 N hydrochloric acid in a 100-mL beaker. Decant and discard the hydrochloric acid, and wash the resin with five 10-mL portions of water, decanting and discarding the liquid following each washing. Place the washed resin in a 125-mL glass-stoppered, conical flask, and add a volume of Oral Solution, nominally equivalent to 250 mg of aminocaproic acid, and 10 mL of water. Insert the stopper in the flask, and shake by mechanical means for 30 min. Transfer the resin slurry to a sintered-glass funnel of medium pore size. Wash with 100 mL of water, filter by applying suction, and discard the washing. Place a beaker under the stem of the funnel, add 10 mL of 1 N hydrochloric acid to the resin,



stir for 4–5 min, and filter by applying suction. Evaporate the filtrate on a steam bath to dryness, dry at 105° for 1 h, and cool.

Acceptance criteria: The residue meets the requirements.

## ASSAY

### PROCEDURE

**Sample solution:** Place an amount nominally equivalent to 250 mg of aminocaproic acid from a volume of Oral Solution in about 80 mL of glacial acetic acid.

#### Titrimetric system

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid in dioxane VS

**Endpoint detection:** Visual

**Analysis:** To the *Sample solution* add 10 drops of a 1-in-500 solution of crystal violet in chlorobenzene. Titrate with *Titrant* to a blue endpoint, and perform a blank determination. Each mL of 0.1 N perchloric acid is equivalent to 13.12 mg of aminocaproic acid ( $C_6H_{13}NO_2$ ).

Acceptance criteria: 95.0%–115.0%

## SPECIFIC TESTS

- PH (791):** 6.0–6.5

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers.
- USP REFERENCE STANDARDS (11)**  
USP Aminocaproic Acid RS

## Aminocaproic Acid Tablets

### DEFINITION

Aminocaproic Acid Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of aminocaproic acid ( $C_6H_{13}NO_2$ ).

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197K)

**Sample:** Triturate 2 Tablets with 10 mL of water, and filter into 100 mL of acetone. Swirl the mixture, and allow to stand for 15 min to complete crystallization. Pass the solution through a sintered-glass filter of medium pore size, and wash the crystals with 25 mL of acetone. Apply vacuum to remove the solvent, dry at 105° for 30 min, and cool. Use the residue.

Acceptance criteria: Meet the requirements

## ASSAY

### PROCEDURE

**Sample solution:** Nominally equivalent to about 500 mg of aminocaproic acid from NLT 20 finely powdered Tablets taken in a beaker in about 100 mL of glacial acetic acid. Heat gently to effect solution, and cool.

#### Titrimetric system

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid in dioxane VS

**Endpoint detection:** Visual

**Analysis:** To the *Sample solution* add 10 drops of a 1-in-500 solution of crystal violet in chlorobenzene. Titrate with *Titrant* to a blue endpoint, and perform a blank determination. Each mL of 0.1 N perchloric acid is equivalent to 13.12 mg of aminocaproic acid ( $C_6H_{13}NO_2$ ).

Acceptance criteria: 95.0%–105.0%

## PERFORMANCE TESTS

### DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Buffer:** Dissolve 6.185 g of boric acid and 7.930 g of potassium chloride in about 1000 mL of water, and add 60 mL of 1.0 N sodium hydroxide. Dilute with water to 2000 mL, and adjust if necessary with 1.0 N sodium hydroxide to a pH of  $9.5 \pm 0.1$ .

**Standard solution:** 0.5 mg/mL of USP Aminocaproic Acid RS in water

**Sample solution:** Filter a portion of the solution under test.

**Blank:** Water

**Analysis:** Into three separate 50-mL volumetric flasks pipet 1 mL each of *Sample solution*, *Standard solution*, and *Blank*. Add 20.0 mL of *Buffer* and 3.0 mL of a freshly prepared 1-in-500 solution of  $\beta$ -naphthoquinone-4-sodium sulfonate to each flask. Swirl to mix, and place the three flasks in a water bath maintained at a temperature of  $65 \pm 5^\circ$  for 45 min. Cool, and dilute the contents of each flask with water to volume. Determine the percentage of the labeled amount of aminocaproic acid ( $C_6H_{13}NO_2$ ) dissolved from absorbances, at the wavelength of maximum absorbance at about 460 nm, from the *Sample solution* in comparison with those from the *Standard solution*, using the *Blank* to set the instrument.

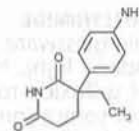
**Tolerances:** NLT 75% (Q) of the labeled amount of aminocaproic acid ( $C_6H_{13}NO_2$ ) is dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers.
- USP REFERENCE STANDARDS (11)**  
USP Aminocaproic Acid RS

## Aminoglutethimide



$C_{13}H_{16}N_2O_2$  232.28  
2,6-Piperidinedione, 3-(4-aminophenyl)-3-ethyl-;  
2-(p-Aminophenyl)-2-ethylglutarimide [125-84-8].

### DEFINITION

Aminoglutethimide contains NLT 98.0% and NMT 102.0% of aminoglutethimide ( $C_{13}H_{16}N_2O_2$ ), calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION (197M)**
- B. ULTRAVIOLET ABSORPTION (197U)**  
**Analytical wavelength:** 242 nm  
**Medium:** Methanol  
**Sample solution:** 10  $\mu$ g/mL  
**Acceptance criteria:** Absorptivities, calculated on a dried basis, differ by NMT 2.0%.

## ASSAY

### PROCEDURE

**Buffer:** Add 120 mL of 0.1 N acetic acid to 100 mL of 0.1 N potassium hydroxide in a 1000-mL volumetric flask, and add 250 mL of water. Adjust by the addition



of either 1 N acetic acid or 1 N potassium hydroxide to a pH of  $5.0 \pm 0.1$ . Dilute with water to volume.

**Mobile phase:** Methanol and Buffer (27:73)

**Diluent:** Methanol and Buffer (1:1)

**Standard solution:** 0.5 mg/mL of USP Aminoglutethimide RS in Diluent

**Sample solution:** 0.5 mg/mL of Aminoglutethimide in Diluent. Pass through a filter of 0.45- $\mu$ m or finer pore size filter, discarding the first 5 mL of the filtrate.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 3.9-mm  $\times$  15-cm; 4- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.3 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** Standard solution

#### Suitability requirements

**Tailing factor:** NMT 1.7

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of aminoglutethimide ( $C_{13}H_{16}N_2O_2$ ) in the portion of Aminoglutethimide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the Sample solution

$r_S$  = peak area from the Standard solution

$C_S$  = concentration of USP Aminoglutethimide RS in the Standard solution (mg/mL)

$C_U$  = concentration of Aminoglutethimide in the Sample solution (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

#### Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm • (Official 1-

Jan-2018)

- **LIMIT OF AZO-AMINOGLUTETHIMIDE**

[NOTE—Use low-actinic glassware. Conduct this test promptly under subdued light. Wear protective gloves resistant to dimethyl sulfoxide to prevent contact with skin. Use shaking, not sonication or heat, to dissolve the USP Azo-aminoglutethimide RS and the Sample.]

**Buffer:** 150 mL of 0.1 N acetic acid and 50 mL of 0.1 N potassium hydroxide, diluted in water to 1000 mL

**Mobile phase:** Dissolve 100 mg of edetate disodium in 350 mL of Buffer. Add 650 mL of methanol, and cool to room temperature. Adjust with glacial acetic acid to a pH of  $5.0 \pm 0.1$ .

**Standard solution:** 0.5  $\mu$ g/mL of USP Azo-aminoglutethimide RS in dimethyl sulfoxide

**Sample solution:** 1 mg/mL of Aminoglutethimide in dimethyl sulfoxide

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 328 nm

**Column:** 3.9-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** Standard solution

#### Suitability requirements

**Tailing factor:** NMT 1.2

**Capacity factor:** 2.0–5.0

**Column efficiency:** NLT 800 theoretical plates

#### Analysis

**Samples:** Standard solution and Sample solution

[NOTE—The aminoglutethimide elutes with the dimethyl sulfoxide.]

Calculate the percentage of azo-aminoglutethimide in the specimen of Aminoglutethimide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Azo-aminoglutethimide RS in the Standard solution ( $\mu$ g/mL)

$C_U$  = concentration of Aminoglutethimide in the Sample solution (mg/mL)

**Acceptance criteria:** NMT 0.03% of 3,3'-(azodi-4,1-phenylene)-3,3'-dimethylbis-[2,6-piperidinedione] (corresponding to azo-aminoglutethimide)

#### • ORGANIC IMPURITIES

**Buffer, Mobile phase, Diluent, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Standard solution:** 10  $\mu$ g/mL of USP *m*-Aminoglutethimide RS in Diluent

**Sample solution:** 1 mg/mL of Aminoglutethimide in Diluent. Pass through a filter of 0.45- $\mu$ m or finer pore size, discarding the first 5 mL of the filtrate.

#### Analysis

**Samples:** Standard solution and Sample solution

[NOTE—The relative retention times for aminoglutethimide and *m*-aminoglutethimide are about 0.8 and 1.0, respectively.]

Calculate the percentage of *m*-aminoglutethimide in the portion of Aminoglutethimide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP *m*-Aminoglutethimide RS in the Standard solution (mg/mL)

$C_U$  = concentration of Aminoglutethimide in the Sample solution (mg/mL)

**Acceptance criteria:** NMT 2.0% of *m*-aminoglutethimide

Calculate the percentage of each peak, other than the main peak and the *m*-aminoglutethimide peak, in the portion of Aminoglutethimide taken, if present:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = response of each peak

$r_T$  = sum of the responses of all the peaks from the Sample solution

**Acceptance criteria:** NMT 1.0% total impurities, other than *m*-aminoglutethimide

#### SPECIFIC TESTS

##### • SULFATE

**Sample solution:** 1 mg/mL in dilute methanol (1 in 20)

**Analysis:** To 100 mL of Sample solution add 1.0 mL of 3 N hydrochloric acid and 2.0 mL of barium chloride TS.



- Acceptance criteria: No turbidity is produced.
- **pH (791)**  
Sample solution: 1 mg/mL in dilute methanol (1 in 20)  
Acceptance criteria: 6.2–7.3
- **Loss on Drying (731)**  
Analysis: Dry at 105° to constant weight.  
Acceptance criteria: NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
USP Aminoglutethimide RS  
USP *m*-Aminoglutethimide RS  
USP Azo-aminoglutethimide RS

**Aminoglutethimide Tablets****DEFINITION**

Aminoglutethimide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of aminoglutethimide ( $C_{13}H_{16}N_2O_2$ ).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197M)**  
Sample: Transfer 500 mg of finely powdered Tablets to a suitable container. Add 25 mL of acetone, mix, and filter. Evaporate the filtrate at room temperature to dryness, and dry the residue under vacuum over silica gel for 2 h.  
Acceptance criteria: Meet the requirements

**ASSAY**• **PROCEDURE**

**Buffer:** Add 120 mL of 0.1 N acetic acid to 100 mL of 0.1 N potassium hydroxide in a 1000-mL volumetric flask, and add 250 mL of water. Adjust by the addition of either 1 N acetic acid or 1 N potassium hydroxide to a pH of  $5.0 \pm 0.1$ . Dilute with water to volume.

**Mobile phase:** Methanol and *Buffer* (27:73)

**Diluent:** Methanol and *Buffer* (1:1)

**Standard solution:** 0.5 mg/mL of USP Aminoglutethimide RS in *Diluent*

**Sample solution:** Nominally 0.5 mg/mL of aminoglutethimide in *Diluent* prepared as follows. Transfer an equivalent to 200 mg of aminoglutethimide, from finely powdered Tablets (NLT 20), to a 200-mL volumetric flask. Add 130 mL of *Diluent*, and sonicate for 5 min. Shake by mechanical means for 30 min, and dilute with *Diluent* to volume. Centrifuge this solution, transfer 25.0 mL of the clear supernatant to a 50-mL volumetric flask, and dilute with *Diluent* to volume. Pass through a filter of 0.45- $\mu$ m or finer pore size, discarding the first 5 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 3.9-mm  $\times$  15-cm; 4- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.3 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.7

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminoglutethimide ( $C_{13}H_{16}N_2O_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Aminoglutethimide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aminoglutethimide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION (711)**

**Medium:** Dilute hydrochloric acid (7 in 1000); 1000 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV

**Analytical wavelength:** 237 nm

**Standard solution:** USP Aminoglutethimide RS in a mixture of dilute hydrochloric acid and pH 7.5 phosphate buffer, having a ratio similar to the *Sample solution*

**Sample solution:** Sample per *Dissolution* (711). Dilute with pH 7.5 phosphate buffer to a concentration that is similar to the *Standard solution*.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** NLT 70% (Q) of the labeled amount of aminoglutethimide ( $C_{13}H_{16}N_2O_2$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES**• **ORGANIC IMPURITIES**

**Buffer, Mobile phase, Diluent, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 10  $\mu$ g/mL of USP *m*-Aminoglutethimide RS in *Diluent*

**Sample solution:** Transfer an equivalent to 200 mg of aminoglutethimide, from finely powdered Tablets (NLT 20), to a 200-mL volumetric flask. Add 130 mL of *Diluent*, and sonicate for 5 min. Shake by mechanical means for 30 min, and dilute with *Diluent* to volume. Pass through a filter of 0.45- $\mu$ m or finer pore size, discarding the first 5 mL of the filtrate.

**System suitability**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for aminoglutethimide and *m*-aminoglutethimide are 0.8 and 1.0, respectively.]

**Suitability requirements**

**Tailing factor:** NMT 1.7, *Sample solution*

**Relative standard deviation:** NMT 2.0%, *Sample solution*

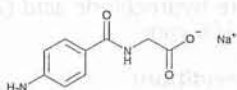


**Analysis****Sample:** *Sample solution*Calculate the percentage of each peak, other than the main peak and the *m*-aminogluthethimide peak, if present in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak response of each impurity in the *Sample solution* $r_T$  = sum of the responses of all of the peaks, excluding that of the *m*-aminogluthethimide peak in the *Sample solution***Acceptance criteria:** NMT 2.0% total impurities, other than *m*-aminogluthethimide**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Aminogluthethimide RS  
USP *m*-Aminogluthethimide RS

**Aminohippurate Sodium Injection** $C_9H_9N_2NaO_3$ Glycine, *N*-(4-aminobenzoyl)-, monosodium salt;  
Monosodium *p*-aminohippurate [94-16-6].

216.17

**DEFINITION**Aminohippurate Sodium Injection is a sterile solution of Aminohippuric Acid in Water for Injection prepared with the aid of Sodium Hydroxide. It contains NLT 95.0% and NMT 105.0% of the labeled amount of aminohippurate sodium ( $C_9H_9N_2NaO_3$ ).**IDENTIFICATION****A.****Sample:** A volume of Injection equivalent to 100 mg of aminohippuric acid**Analysis:** Dilute the *Sample* with water to 50 mL. To 5 mL of this solution add 0.5 mL of 3 N hydrochloric acid, 0.5 mL of sodium nitrite solution (1 in 10), and a solution of 0.20 g of 2-naphthol in 10 mL of 6 N ammonium hydroxide.**Acceptance criteria:** A red color is produced.**B.****Sample:** A volume of Injection equivalent to about 200 mg of aminohippurate sodium**Analysis:** In the order named, add to the *Sample* 2 mL of potassium iodide TS, 10 mL of water, and 5 mL of sodium hypochlorite TS.**Acceptance criteria:** A red color is produced.

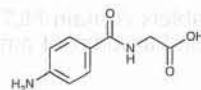
- **C. IDENTIFICATION TESTS—GENERAL, Sodium (191):** Meets the requirements of the flame test

**ASSAY****PROCEDURE****Sample:** A volume of Injection equivalent to 1 g of aminohippurate sodium**Analysis:** Transfer the *Sample* to a 200-mL volumetric flask, and dilute with water to volume. Transfer 50.0 mL of the solution to a suitable container, and add 5 mL of hydrochloric acid. Proceed as directed in *Nitrite Titration* (451), beginning with "cool to about 15°". Each mL of0.1 M sodium nitrite is equivalent to 21.62 mg of aminohippurate sodium ( $C_9H_9N_2NaO_3$ ).**Acceptance criteria:** 95.0%–105.0%**SPECIFIC TESTS**

- **PH (791):** 6.7–7.6
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.04 USP Endotoxin Unit/mg of aminohippurate sodium
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type 1 glass.
- **USP REFERENCE STANDARDS (11)**  
USP Endotoxin RS

**Aminohippuric Acid** $C_9H_9N_2O_3$ Glycine, *N*-(4-aminobenzoyl)-;  
*p*-Aminohippuric acid [61-78-9].

194.19

**DEFINITION**Aminohippuric Acid contains NLT 98.0% and NMT 100.5% of aminohippuric acid ( $C_9H_9N_2O_3$ ), calculated on the dried basis.**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B.**

**Sample:** 10 mg of Aminohippuric Acid**Analysis:** Dissolve the *Sample* in 5 mL of water, add 0.5 mL of 3 N hydrochloric acid, 0.5 mL of sodium nitrite solution (1 in 10), and a solution of 0.20 g of 2-naphthol in 10 mL of 6 N ammonium hydroxide.**Acceptance criteria:** A red color is produced.**ASSAY****PROCEDURE****Sample:** 150 mg of Aminohippuric Acid**Analysis:** To the *Sample* add 5 mL of hydrochloric acid and 50 mL of water. Proceed as directed in *Nitrite Titration* (451), beginning with "stir until dissolved." Each mL of 0.1 M sodium nitrite is equivalent to 19.42 mg of aminohippuric acid ( $C_9H_9N_2O_3$ ).**Acceptance criteria:** 98.0%–100.5% on the dried basis**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 0.25%

**Delete the following:**

- **HEAVY METALS, Method II (231):** NMT 0.001% (Official 1-  
Jan-2018)

**SPECIFIC TESTS**

- **LOSS ON DRYING (731)**  
**Analysis:** Dry at 105° for 2 h.  
**Acceptance criteria:** NMT 0.25%

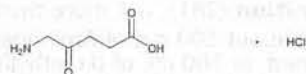
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.



- **USP REFERENCE STANDARDS** (11)  
USP Aminohippuric Acid RS

## Aminolevulinic Acid Hydrochloride



$C_5H_9NO_3 \cdot HCl$  167.59  
5-Aminolevulinic acid hydrochloride;  
5-Amino-4-oxopentanoic acid hydrochloride [5451-09-2].

### DEFINITION

Aminolevulinic Acid Hydrochloride contains NLT 98.0% and NMT 102.0% of aminolevulinic acid hydrochloride ( $C_5H_9NO_3 \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION**, (197K) or (197A)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL**, *Chloride* (191): Meets the requirements

### ASSAY

#### PROCEDURE

**Solution A:** Water adjusted with 2 M sulfuric acid to a pH of 2.2

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Methanol (%)
0	95	5
2	95	5
6	60	40
8	60	40
9	95	5
23	95	5

**Diluent:** Methanol and *Solution A* (1:3)

**Standard solution:** 4 mg/mL of USP Aminolevulinic Acid Hydrochloride RS in *Diluent*

**Sample solution:** 4 mg/mL of Aminolevulinic Acid Hydrochloride in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 2.1-mm × 10-cm; 1.7-μm packing L1

**Flow rate:** 0.4 mL/min

**Injection volume:** 5 μL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.6

**Relative standard deviation:** NMT 0.73%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminolevulinic acid hydrochloride ( $C_5H_9NO_3 \cdot HCl$ ) in the portion of Aminolevulinic Acid Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Aminolevulinic Acid Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aminolevulinic Acid Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.3%

#### ORGANIC IMPURITIES

**Mobile phase, Diluent, and Chromatographic system:**

Proceed as directed in the *Assay*.

**Standard solution:** 0.04 mg/mL each of USP Aminolevulinic Acid Hydrochloride RS, USP Aminolevulinic Acid Related Compound A RS, and USP Aminolevulinic Acid Related Compound B RS in *Diluent*

**Sample solution:** 40 mg/mL of Aminolevulinic Acid Hydrochloride in *Diluent*

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 10%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminolevulinic acid related compound A or aminolevulinic acid related compound B in the portion of Aminolevulinic Acid Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of the corresponding USP Reference Standard from the *Standard solution*

$C_S$  = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aminolevulinic Acid Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity eluting before aminolevulinic acid related compound A in the portion of Aminolevulinic Acid Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity eluting before aminolevulinic acid related compound A from the *Sample solution*

$r_S$  = peak response of aminolevulinic acid from the *Standard solution*

$C_S$  = concentration of USP Aminolevulinic Acid Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aminolevulinic Acid Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity eluting after aminolevulinic acid related compound A in the portion of Aminolevulinic Acid Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity eluting after aminolevulinic acid related compound A from the *Sample solution*

$r_S$  = peak response of aminolevulinic acid related compound A from the *Standard solution*

$C_S$  = concentration of USP Aminolevulinic Acid Related Compound A RS in the *Standard solution* (mg/mL)



$C_U$  = concentration of Aminolevulinic Acid Hydrochloride in the Sample solution (mg/mL)

**Acceptance criteria:** See Table 2. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminolevulinic acid	1.0	—
Aminolevulinic acid related compound A	7.8	0.15
Aminolevulinic acid related compound B	12.0	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

### SPECIFIC TESTS

#### • pH (791)

**Sample solution:** 10 mg/mL in carbon dioxide-free water

**Acceptance criteria:** 2.5–2.9

#### • Loss on Drying (731)

**Sample:** 1 g

**Analysis:** Dry the Sample over phosphorous pentoxide under vacuum at 100°–105° for 5 h.

**Acceptance criteria:** NMT 0.5%

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at room temperature.

#### • USP REFERENCE STANDARDS (11)

USP Aminolevulinic Acid Hydrochloride RS

USP Aminolevulinic Acid Related Compound A RS  
3,3'-(Pyrazine-2,5-diyl)dipropionic acid.

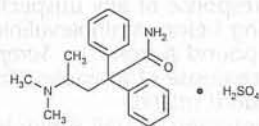
$C_{10}H_{12}N_2O_4$  224.22

USP Aminolevulinic Acid Related Compound B RS

Methyl 5-(1,3-dioxoisindolin-2-yl)-4-oxopentanoate.

$C_{14}H_{13}NO_5$  275.26

## Aminopentamide Sulfate



$C_{19}H_{24}N_2O \cdot H_2SO_4$  394.49

$\alpha$ -[2-(Dimethylamino)propyl]- $\alpha$ -phenylbenzeneacetamide sulfate [60-46-8].

» Aminopentamide Sulfate contains not less than 95.0 percent and not more than 103.0 percent of  $C_{19}H_{24}N_2O \cdot H_2SO_4$ .

**Packaging and storage—**Preserve in tight containers, and store at controlled room temperature.

**Labeling—**Label it to indicate that it is for veterinary use only.

#### USP Reference standards (11)—

USP Aminopentamide Sulfate RS

**Clarity and color of solution—**Dissolve 0.5 g in 10 mL of water; the solution is clear and colorless.

### Identification—

**A: Infrared Absorption (197K).**

**B:** It meets the requirements of the tests for Sulfate (191).

**Melting range (741):** between 179° and 186°.

**pH (791):** between 1.2 and 3.0, in a solution (2.5 in 100).

**Loss on drying (731)—**Dry it at 105° for 4 hours; it loses not more than 4.4% of its weight.

**Residue on ignition (281):** not more than 0.5%.

**Assay—**Dissolve about 500 mg of Aminopentamide Sulfate, accurately weighed, in 100 mL of dimethylformamide in a suitable container. Add 5 drops of thymol blue TS, and titrate with 0.1 N lithium methoxide VS in toluene to a deep blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N lithium methoxide is equivalent to 19.72 mg of  $C_{19}H_{24}N_2O \cdot H_2SO_4$ .

## Aminopentamide Sulfate Injection

» Aminopentamide Sulfate Injection is a sterile solution of Aminopentamide Sulfate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aminopentamide sulfate ( $C_{19}H_{24}N_2O \cdot H_2SO_4$ ).

**Packaging and storage—**Preserve in tight, single-dose or multiple-dose Containers for Injections, as described in Packaging and Storage Requirements (659), Injection Packaging. Store at controlled room temperature.

**Labeling—**Label Injection to indicate that it is for veterinary use only.

#### USP Reference standards (11)—

USP Aminopentamide Sulfate RS

USP Endotoxin RS

**Identification—**Transfer 10 mL of the Injection to a separator, add sodium hydroxide TS until alkaline to litmus, and extract with 25 mL of chloroform. Transfer a few drops of the chloroform extract to a KRS-5 plate, and allow to dry. Record the IR absorption spectrum by the attenuated total reflectance technique (see Mid-Infrared Spectroscopy (854)). The spectrum thus obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Aminopentamide Sulfate RS, concomitantly measured.

**Bacterial Endotoxins Test (85)—**It contains not more than 25 USP Endotoxin Units per mg of aminopentamide sulfate.

**Sterility Tests (71)—**It meets the requirements when tested as directed for Membrane Filtration under Test for Sterility of the Product to be Examined.

**pH (791):** between 2.5 and 4.5.

**Other requirements—**It meets the requirements under Injections and Implanted Drug Products (1).

### Assay—

**Mobile phase—**Transfer 14.4 g of sodium lauryl sulfate to a 500-mL volumetric flask, add 100 mL of glacial acetic acid, dilute with water to volume, mix, and pass through a filter having a 0.5- $\mu$ m or finer porosity. Transfer 50 mL of this solution to a 1000-mL volumetric flask, add 350 mL of methanol and 350 mL of acetonitrile, dilute with water to volume, and mix. Filter and degas before use. Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Standard preparation—**Quantitatively dissolve an accurately weighed quantity of USP Aminopentamide Sulfate RS in water to obtain a solution having a known concentration



equivalent to the labeled concentration of aminopentamide sulfate in the Injection.

**Assay preparation**—Use the undiluted Injection.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of aminopentamide sulfate ( $C_{19}H_{24}N_2O \cdot H_2SO_4$ ) in each mL of the Injection taken by the formula:

$$C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Aminopentamide Sulfate RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the aminopentamide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aminopentamide Sulfate Tablets

» Aminopentamide Sulfate Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of aminopentamide sulfate ( $C_{19}H_{24}N_2O \cdot H_2SO_4$ ).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Labeling**—Label Tablets to indicate that they are for veterinary use only.

**USP Reference standards** (11)—  
USP Aminopentamide Sulfate RS

**Identification**—Transfer a portion of ground Tablet powder, equivalent to about 2 mg of aminopentamide, to a separator, add 20 mL of water and 3 mL of 10 N sodium hydroxide, and mix. Extract with two 20-mL portions of methylene chloride, and evaporate the combined methylene chloride extracts to a volume of about 0.5 mL. Transfer a few drops of the chloroform concentrate to a KRS-5 plate, and allow to dry. Record the IR absorption spectrum by the attenuated total reflectance technique (see *Mid-Infrared Spectroscopy* (854)). The spectrum thus obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Aminopentamide Sulfate RS, concomitantly measured.

**Disintegration** (701): not more than 10 minutes, simulated gastric fluid TS being substituted for water in the test.

**Uniformity of dosage units** (905): meet the requirements.

**Loss on drying** (731)—Dry about 1 g of powdered Tablets, accurately weighed, in vacuum at a pressure of 5 mm of mercury or less at 60° for 3 hours: it loses not more than 4.0% of its weight.

**Assay**—

**Mobile phase**—Transfer 14.4 g of sodium lauryl sulfate to a 500-mL volumetric flask, add 100 mL of glacial acetic acid, dilute with water to volume, mix, and pass through a filter having a 0.5-µm or finer porosity. Transfer 50 mL of this solution to a 1000-mL volumetric flask, add 350 mL of

methanol and 350 mL of acetonitrile, dilute with water to volume, and mix. Filter and degas before use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Aminopentamide Sulfate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.02 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 10 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.2 mg of aminopentamide, to a suitable flask. Add 10.0 mL of *Mobile phase*, sonicate for 5 minutes, and stir by mechanical means for about 10 minutes. Pass this mixture through a filter having a 0.5-µm or finer porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

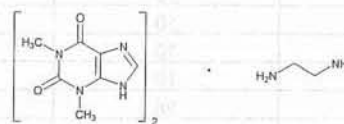
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1, and is maintained at a constant temperature of about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 900 theoretical plates; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of aminopentamide sulfate ( $C_{19}H_{24}N_2O \cdot H_2SO_4$ ) in the portion of Tablets taken by the formula:

$$10C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Aminopentamide Sulfate RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the aminopentamide peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aminophylline



$C_{16}H_{24}N_{10}O_4$  420.43

$C_{16}H_{24}N_{10}O_4 \cdot 2H_2O$  456.46

1*H*-Purine-2,6-dione, 3,7-dihydro-1,3-dimethyl-, compd.

with 1,2-ethanediamine (2:1);

Theophylline compound with ethylenediamine (2:1).

Anhydrous [317-34-0].

Dihydrate [5897-66-5].

### DEFINITION

Aminophylline is anhydrous or contains NMT two molecules of water of hydration. It contains NLT 84.0% and NMT 87.4% of anhydrous theophylline ( $C_7H_8N_4O_2$ ), calculated on the anhydrous basis.

### IDENTIFICATION

#### Change to read:

- **A. INFRARED ABSORPTION** (197): [NOTE—Methods described in (197K) or (197A) may be used.]▲USP40



**Sample:** 500 mg of Aminophylline

**Analysis:** Dissolve the *Sample* in 20 mL of water, add, with constant stirring, 1 mL of 3 N hydrochloric acid, filter (retain the filtrate), wash the precipitate with small portions of cold water, and dry at 105° for 1 h.

**Acceptance criteria:** <sup>▲</sup>The IR spectrum of theophylline so obtained corresponds to that of USP Theophylline RS. <sup>▲</sup>USP40

#### Change to read:

- **B.** <sup>▲</sup>The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. <sup>▲</sup>USP40

- **C.** **Sample:** The filtrate obtained in *Identification A*.

**Analysis:** To the *Sample* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline. Shake by mechanical means for 10 min, add 5 mL of 3 N hydrochloric acid to acidify, chill, collect the precipitated disulfonamide of ethylenediamine, wash with water, recrystallize from water, and dry at 105° for 1 h.

**Acceptance criteria:** The dried precipitate melts at 164°–171°.

#### ASSAY

#### Change to read:

##### • PROCEDURE

<sup>▲</sup>**Solution A:** 10 mM ammonium acetate prepared as follows. Transfer 770.8 mg of ammonium acetate to a 1-L volumetric flask, and dissolve in water to 80% of the flask volume. Adjust with glacial acetic acid to a pH of 5.5, and dilute with water to volume. Pass through a suitable filter of 0.2-μm pore size.

**Solution B:** Methanol

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

**Impurity stock solution:** 25 μg/mL of USP Theophylline Related Compound F RS in water

**System suitability solution:** 0.8 mg/mL of USP Theophylline RS and 1 μg/mL of USP Theophylline Related Compound F RS in water prepared as follows. Transfer 21 mg of USP Theophylline RS to a 25-mL volumetric flask and add 15 mL of water. Sonicate to dissolve, add 1 mL of *Impurity stock solution*, and dilute with water to volume.

**Standard solution:** 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve as needed.

**Sample solution:** 0.2 mg/mL of Aminophylline in water

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 2.1-mm × 10-cm; 1.7-μm packing L1

**Column temperature:** 40°

**Flow rate:** 0.4 mL/min

**Injection volume:** 1 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) in the portion of Aminophylline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response of theophylline from the *Sample solution*

*r<sub>S</sub>* = peak response of theophylline from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = concentration of Aminophylline in the *Sample solution* (mg/mL) <sup>▲</sup>USP40

**Acceptance criteria:** 84.0%–87.4% of theophylline on the anhydrous basis

#### OTHER COMPONENTS

#### Change to read:

##### • <sup>▲</sup>CONTENT OF ETHYLENEDIAMINE <sup>▲</sup>USP40

**Sample:** 500 mg of Aminophylline

**Diluent:** Water

#### Titrimetric system

**Mode:** Direct titration

**Titrant:** 0.1 N hydrochloric acid VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 30 mL of *Diluent*, add methyl orange TS, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>).

**Acceptance criteria:** 157–175 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>) per gram of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) found in the *Assay*

#### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.15%

#### Add the following:

##### • <sup>▲</sup>ORGANIC IMPURITIES

**Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard stock solution:** 25.0 μg/mL each of USP Caffeine RS, USP Theophylline RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, USP Theophylline Related Compound D RS, and USP Theophylline Related Compound F RS in water

**Standard solution:** 1.0 μg/mL each of USP Caffeine RS, USP Theophylline RS, USP Theophylline Related Compound B RS, USP Theophylline Related Compound C RS, USP Theophylline Related Compound D RS, and USP Theophylline Related Compound F RS in water, from *Standard stock solution*



**Sample solution:** 1.0 mg/mL of Aminophylline in water

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

**Relative standard deviation:** NMT 3.0% for each peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of caffeine, theophylline related compound B, theophylline related compound C, theophylline related compound D, and theophylline related compound F in the portion of Aminophylline taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of caffeine, theophylline related compound B, theophylline related compound C, theophylline related compound D, or theophylline related compound F from the *Sample solution*

$r_s$  = peak response of the corresponding Reference Standard from the *Standard solution*

$C_s$  = concentration of the corresponding Reference Standard in the *Standard solution* (mg/mL)

$C_u$  = concentration of Aminophylline in the *Sample solution* (mg/mL)

Calculate the percentage of dimethyl uric acid, theobromine, and any other individual unspecified impurity in the portion of Aminophylline taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of dimethyl uric acid, theobromine, or any other individual unspecified impurity from the *Sample solution*

$r_s$  = peak response of theophylline from the *Standard solution*

$C_s$  = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Aminophylline in the *Sample solution* (mg/mL)

$F$  = relative response factor

**Acceptance criteria:** See Table 2. Disregard peaks less than 0.05%.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Theophylline related compound C	0.36	—	0.10
Theophylline related compound B	0.63	—	0.10
Theophylline related compound D	0.69	—	0.10
Dimethyl uric acid <sup>a</sup>	0.76	0.55	0.10
Theobromine <sup>b</sup>	0.82	1.0	0.10
Theophylline	1.0	—	—
Theophylline related compound F	1.09	—	0.10
Caffeine	1.20	—	0.10

<sup>a</sup> 1,3-Dimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione.

<sup>b</sup> 3,7-Dihydro-3,7-dimethylpurine-2,6(1H)-dione.

**Table 2 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.3

<sup>a</sup> 1,3-Dimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione.

<sup>b</sup> 3,7-Dihydro-3,7-dimethylpurine-2,6(1H)-dione.

▲USP40

## SPECIFIC TESTS

### • WATER DETERMINATION (921), Method I

**Sample:** 1.5 g of Aminophylline

**Solvent:** 50 mL of chloroform and anhydrous methanol (50:50) in place of anhydrous methanol

**Acceptance criteria**

**Anhydrous:** NMT 0.75%

**Hydrous:** NMT 7.9%

## ADDITIONAL REQUIREMENTS

### Change to read:

- **PACKAGING AND STORAGE:** Preserve in tight containers.  
▲Store at controlled room temperature.▲USP40
- **LABELING:** Label it to indicate whether it is anhydrous or hydrous, and also to state the content of anhydrous theophylline.

### Change to read:

### • USP REFERENCE STANDARDS (11)

▲USP Caffeine RS▲USP40

USP Theophylline RS

▲USP Theophylline Related Compound B RS

3-Methyl-1H-purine-2,6-dione;

Also known as 3-Methyl-3,7-dihydro-1H-purine-2,6-dione.

$C_8H_8N_4O_2$  166.14

USP Theophylline Related Compound C RS

N-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide.

$C_7H_{10}N_4O_3$  198.18

USP Theophylline Related Compound D RS

N-Methyl-5-(methylamino)-1H-imidazole-4-carboxamide.

$C_6H_{10}N_4O$  154.17

USP Theophylline Related Compound F RS

7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione.

$C_9H_{12}N_4O_3$  224.22▲USP40

## Aminophylline Injection

### DEFINITION

Aminophylline Injection is a sterile solution of Aminophylline in Water for Injection, or is a sterile solution of Theophylline in Water for Injection prepared with the aid of Ethylenediamine. It contains, in each mL, an amount of aminophylline ( $C_{16}H_{24}N_{10}O_4$ ) equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of anhydrous theophylline ( $C_7H_8N_4O_2$ ).

Aminophylline Injection may contain an excess of Ethylenediamine, but no other substance may be added for the purpose of pH adjustment.



[NOTE—Do not use the Injection if crystals have separated.]

## IDENTIFICATION

### A.

**Analysis:** Dilute a volume of Injection equivalent to 500 mg of aminophylline with water to 20 mL, and add, with constant stirring, 1 mL of 3 N hydrochloric acid or enough to completely precipitate the theophylline, and filter. To the filtrate add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline. Shake by mechanical means for 10 min, add 5 mL of 3 N hydrochloric acid to acidify, chill, collect the precipitated disulfonamide of ethylenediamine, wash with water, recrystallize from water, and dry at 105° for 1 h.

**Acceptance criteria:** The precipitate melts at 164°–171°.

### Change to read:

### B. <sup>▲</sup>INFRARED ABSORPTION (197K) <sup>▲</sup>USP40

**Analysis:** Wash the precipitated theophylline from Identification A with small portions of cold water, and dry at 105° for 1 h.

**Acceptance criteria:** <sup>▲</sup>The IR spectrum of theophylline so obtained corresponds to that of USP Theophylline RS. <sup>▲</sup>USP40

### Change to read:

- **C.** <sup>▲</sup>The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. <sup>▲</sup>USP40

## ASSAY

### Change to read:

### PROCEDURE

**▲Solution A:** 10 mM ammonium acetate prepared as follows. Transfer 770.8 mg of ammonium acetate to a 1-L volumetric flask, and dissolve in water to 80% of the flask volume. Adjust with glacial acetic acid to a pH of 5.5 and dilute with water to volume. Pass through a suitable filter of 0.2-μm pore size.

**Solution B:** Methanol

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

**Impurity stock solution:** 25 μg/mL of USP Theophylline Related Compound F RS in water

**System suitability solution:** 0.8 mg/mL of USP Theophylline RS and 1 μg/mL of USP Theophylline Related Compound F RS in water prepared as follows. Transfer 21 mg of USP Theophylline RS to a 25-mL volumetric flask, and add 15 mL of water. Sonicate to dissolve, add 1 mL of *Impurity stock solution*, and dilute with water to volume.

**Standard solution:** 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve as needed.

**Sample solution:** Nominally 0.17 mg/mL of anhydrous theophylline in water prepared as follows. Transfer

8.5 mg of anhydrous theophylline from a volume of Injection to a 50-mL volumetric flask. Dissolve and dilute with water to volume.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 2.1-mm × 10-cm; 1.7-μm packing L1

**Column temperature:** 40°

**Flow rate:** 0.4 mL/min

**Injection volume:** 1 μL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of theophylline from the *Sample solution*

$r_S$  = peak response of theophylline from the *Standard solution*

$C_S$  = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of theophylline in the *Sample solution* (mg/mL) <sup>▲</sup>USP40

**Acceptance criteria:** 93.0%–107.0%

## OTHER COMPONENTS

### • CONTENT OF ETHYLENEDIAMINE

**Sample:** A volume of Injection equivalent to 500 mg of aminophylline

**Diluent:** Water

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N hydrochloric acid VS

**Endpoint detection:** Visual

**Analysis:** If necessary, dilute the *Sample* with *Diluent* to 30 mL, add methyl orange TS, and titrate with *Titrant*. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>).

**Acceptance criteria:** 166–192 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>) per gram of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) found in the *Assay*

## IMPURITIES

### Add the following:

### ▲• ORGANIC IMPURITIES

**Solution A, Solution B, Mobile phase, Impurity stock solution, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 2.0 μg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS in water

**Sample solution:** Nominally 1.0 mg/mL of anhydrous aminophylline in water prepared as follows. Transfer 25 mg of anhydrous aminophylline from a volume of Injection to a 25-mL volumetric flask. Dissolve and dilute with water to volume.

### System suitability

**Samples:** *System suitability solution* and *Standard solution*



[NOTE—See Table 2 for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

**Relative standard deviation:** NMT 3.0% for each peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of theophylline related compound D in the portion of injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of theophylline related compound D from the *Sample solution*

$r_s$  = peak response of theophylline related compound D from the *Standard solution*

$C_s$  = concentration of USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of aminophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of any other individual unspecified degradation product from the *Sample solution*

$r_s$  = peak response of theophylline from the *Standard solution*

$C_s$  = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of aminophylline in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 2. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound C <sup>a,b</sup>	0.36	—
Theophylline related compound B <sup>a,c</sup>	0.63	—
Theophylline related compound D	0.69	0.2
Dimethyl uric acid <sup>a,d</sup>	0.76	—
Theobromine <sup>a,e</sup>	0.82	—
Theophylline	1.0	—
Theophylline related compound F <sup>a</sup>	1.09	—
Caffeine <sup>a</sup>	1.20	—
Any other individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

<sup>a</sup> Process impurity included for identification only and not to be included in the calculation of total degradation products.

<sup>b</sup> N-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide.

<sup>c</sup> 3-Methyl-1H-purine-2,6-dione.

<sup>d</sup> 1,3-Dimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione.

<sup>e</sup> 3,7-Dihydro-3,7-dimethylpurine-2,6(1H)-dione.

▲USP40

#### SPECIFIC TESTS

• **PH (791):** 8.6–9.0

• **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections

- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 1.0 USP Endotoxin Unit/mg of aminophylline.

#### ADDITIONAL REQUIREMENTS

##### Change to read:

- **PACKAGING AND STORAGE:** Preserve in single-dose containers from which carbon dioxide has been excluded, preferably of Type I glass, protected from light. ▲Store at controlled room temperature.▲USP40
- **LABELING:** Label the Injection to state the content of anhydrous theophylline.

##### Change to read:

#### USP REFERENCE STANDARDS (11)

USP Endotoxin RS

USP Theophylline RS

▲USP Theophylline Related Compound D RS

N-Methyl-5-(methylamino)-1H-imidazole-4-carboxamide.

C<sub>6</sub>H<sub>10</sub>N<sub>4</sub>O 154.17

USP Theophylline Related Compound F RS

7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione.

C<sub>9</sub>H<sub>12</sub>N<sub>4</sub>O<sub>3</sub> 224.22▲USP40

## Aminophylline Oral Solution

#### DEFINITION

Aminophylline Oral Solution is an aqueous solution of Aminophylline, prepared with the aid of Ethylenediamine. It contains an amount of aminophylline (C<sub>16</sub>H<sub>24</sub>N<sub>10</sub>O<sub>4</sub>) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>).

Aminophylline Oral Solution may contain an excess of ethylenediamine, but no other substance may be added for the purpose of pH adjustment.

#### IDENTIFICATION

##### Change to read:

#### A. ▲INFRARED ABSORPTION (197K)▲USP40

**Analysis:** Transfer a volume of Oral Solution equivalent to 500 mg of aminophylline to a suitable container and add, with constant stirring, 1 mL of 3 N hydrochloric acid or enough to completely precipitate the theophylline. Filter (retain the filtrate), wash the precipitate with small portions of cold water until free from chloride, and dry at 105° for 1 h.

**Acceptance criteria:** ▲The IR spectrum of theophylline so obtained matches that of USP Theophylline RS.▲USP40

#### B.

**Analysis:** To the filtrate obtained in Identification A add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline. Shake by mechanical means for 10 min, add 5 mL of 3 N hydrochloric acid to acidify, chill, collect the precipitated disulfonamide of ethylenediamine, wash with water, recrystallize from water, and dry at 105° for 1 h.

**Acceptance criteria:** The dried precipitate melts at 164°–171°.



## ASSAY

## Change to read:

## • PROCEDURE

**▲Solution A:** 10 mM ammonium acetate prepared as follows. Transfer an appropriate amount of ammonium acetate to a volumetric flask and dissolve in water (about 80% of the flask volume). Adjust with glacial acetic acid to a pH of 5.5 and dilute with water to volume. Pass through a suitable filter of 0.2-μm pore size.

**Solution B:** Methanol

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

**Impurity stock solution:** 25 μg/mL of USP Theophylline Related Compound F RS in water

**System suitability solution:** 0.8 mg/mL of USP Theophylline RS and 2 μg/mL of USP Theophylline Related Compound F RS in water prepared as follows. Transfer 1 mg of USP Theophylline RS to a 25-mL volumetric flask and add 15 mL of water. Sonicate to dissolve, add 2 mL of *Impurity stock solution*, and dilute with water to volume.

**Standard solution:** 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve, as needed.

**Sample solution:** Nominally 0.17 mg/mL of anhydrous theophylline in water prepared as follows. Transfer an appropriate amount of anhydrous theophylline from a volume of Oral Solution to a suitable volumetric flask. Dissolve and dilute with water to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 2.1-mm × 10-cm; 1.7-μm packing L1

**Column temperature:** 40°

**Flow rate:** 0.4 mL/min

**Injection volume:** 1 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*▲USP40

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of theophylline in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**OTHER COMPONENTS**

## • CONTENT OF ETHYLENEDIAMINE

**Sample:** A volume of Oral Solution equivalent to 500 mg of aminophylline

**Diluent:** Water

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N hydrochloric acid VS

**Endpoint detection:** Visual

**Analysis:** If necessary, dilute the *Sample* with *Diluent* to 30 mL, add methyl orange TS, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>).

**Acceptance criteria:** 176–283 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>) per gram of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) found in the Assay

**IMPURITIES**

## Add the following:

## ▲• ORGANIC IMPURITIES

**Solution A, Solution B, Mobile phase, Impurity stock solution, System suitability solution, and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution:** 2.0 μg/mL each of USP Theophylline RS and USP Theophylline Related Compound D RS in water

**Sample solution:** Nominally 1.0 mg/mL of anhydrous aminophylline in water prepared as follows. Transfer an appropriate amount of anhydrous aminophylline from a volume of Oral Solution to a suitable volumetric flask. Dissolve and dilute with water to volume.

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for relative retention times.]

**Suitability requirements**

**Resolution:** NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

**Relative standard deviation:** NMT 3.0% for each peak present in the *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of theophylline related compound D in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of theophylline related compound D from the *Sample solution*

$r_S$  = peak response of theophylline related compound D from the *Standard solution*

$C_S$  = concentration of USP Theophylline Related Compound D RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of anhydrous theophylline in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified degradation product in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any other individual unspecified degradation product from the *Sample solution*

$r_S$  = peak response of theophylline from the *Standard solution*

$C_S$  = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)



$C_U$  = nominal concentration of anhydrous theophylline in the *Sample solution* (mg/mL)  
**Acceptance criteria:** See Table 2. Disregard peaks less than 0.086%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound C <sup>a,b</sup>	0.36	—
Theophylline related compound B <sup>a,c</sup>	0.63	—
Theophylline related compound D	0.69	0.2
Dimethyl uric acid <sup>a,d</sup>	0.76	—
Theobromine <sup>a,e</sup>	0.82	—
Theophylline	1.0	—
Theophylline related compound F <sup>a</sup>	1.09	—
Caffeine <sup>a</sup>	1.20	—
Any other individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

<sup>a</sup> Process impurity included for identification only and not to be included in the calculation of total degradation products.

<sup>b</sup> *N*-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide.

<sup>c</sup> 3-Methyl-1*H*-purine-2,6-dione.

<sup>d</sup> 1,3-Dimethyl-7,9-dihydro-1*H*-purine-2,6,8(3*H*)-trione.

<sup>e</sup> 3,7-Dihydro-3,7-dimethylpurine-2,6(1*H*)-dione.

▲USP40

### SPECIFIC TESTS

- **pH (791):** 8.5–9.7

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Oral Solution to state the content of anhydrous theophylline.

### Change to read:

#### • USP REFERENCE STANDARDS (11)

USP Theophylline RS

▲USP Theophylline Related Compound D RS

*N*-Methyl-5-(methylamino)-1*H*-imidazole-4-carboxamide.

$C_6H_{10}N_4O$  154.17

USP Theophylline Related Compound F RS

7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione.

$C_9H_{12}N_4O_3$  224.22▲USP40

## Aminophylline Rectal Solution

### DEFINITION

Aminophylline Rectal Solution is an aqueous solution of Aminophylline, prepared with the aid of Ethylenediamine. It contains an amount of aminophylline equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline ( $C_7H_8N_4O_2$ ).

Rectal Solution may contain an excess of ethylenediamine, but no other substance may be added for the purpose of pH adjustment.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Sample:** Dilute an amount of Rectal Solution, equivalent to 500 mg of aminophylline, with water to 20 mL. Add, with constant stirring, 1 mL of 3 N hydrochloric

acid or enough to precipitate the theophylline completely, and filter (save the filtrate). Wash the precipitate with a small portion of cold water until free from chloride, and dry at 105° for 4 h.

**Acceptance criteria:** The dried precipitate meets the requirements.

#### • B.

**Analysis:** To the filtrate obtained in *Identification* test A add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline, shake by mechanical means for 10 min, and add 5 mL of 3 N hydrochloric acid to acidify. Chill, collect the precipitated disulfonamide of ethylenediamine, and wash with water. Recrystallize the washed precipitate from water, and dry at 105° for 1 h.

**Acceptance criteria:** The dried precipitate melts at 164°–171°.

### ASSAY

#### • PROCEDURE

**Standard solution:** 8 µg/mL of USP Theophylline RS in dilute hydrochloric acid (1:100)

**Sample solution:** Pipet a volume of Rectal Solution equivalent to 500 mg of aminophylline into a 500-mL volumetric flask, and dilute with water to volume. Pipet 5 mL of this solution to a second 500-mL volumetric flask, add 50-mL of dilute hydrochloric acid (1:10), and dilute with water to volume.

#### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** About 270 nm

**Cell:** 1 cm

**Blank:** Dilute hydrochloric acid (1:100)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anhydrous theophylline ( $C_7H_8N_4O_2$ ) in the portion of Rectal Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Theophylline RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of theophylline in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

### OTHER COMPONENTS

#### • CONTENT OF ETHYLENEDIAMINE

**Sample solution:** Measure a volume of Rectal Solution, equivalent to 500 mg of aminophylline, and dilute with water if necessary to make 30 mL.

#### Titrimetric system

**Mode:** Direct titration

**Titrant:** 0.1 N hydrochloric acid VS

**Endpoint detection:** Visual

**Analysis:** Add methyl orange TS to the *Sample solution*, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylene diamine ( $C_2H_8N_2$ ).

**Acceptance criteria:** 218–267 mg of ethylenediamine ( $C_2H_8N_2$ ) per g of theophylline ( $C_7H_8N_4O_2$ ) found in the Assay

### SPECIFIC TESTS

- **pH (791):** 9.0–9.5

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, single-dose or multiple-dose containers at controlled room temperature.
- **LABELING:** Label the Rectal Solution to state the content of anhydrous theophylline.



• **USP REFERENCE STANDARDS (11)**

USP Theophylline RS

## Aminophylline Suppositories

### DEFINITION

Aminophylline Suppositories contain an amount of aminophylline equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous theophylline ( $C_7H_8N_4O_2$ ).

### IDENTIFICATION

- **A.**  
**Analysis:** Evaporate on a steam bath a portion of *Sample stock solution* from the *Assay*, equivalent to 500 mg of aminophylline, to about one-half its volume. Adjust with 1 N sodium hydroxide to a pH of 7.0, chill, and filter the crystals of theophylline. Retain the filtrate, free from washings. Wash the crystals of theophylline with small portions of ice-cold water, and dry at 105° for 1 h.  
**Acceptance criteria:** The recrystallized theophylline melts at 270°–274°.
- **B.**  
**Analysis:** Transfer 10 mg of the dried precipitate from *Identification test A* to a porcelain dish, and add 1 mL of hydrochloric acid and 100 mg of potassium chlorate. Evaporate on a steam bath to dryness, and invert the dish over a vessel containing a few drops of 6 N ammonium hydroxide.  
**Acceptance criteria:** The residue acquires a purple color, which is destroyed by solutions of fixed alkalis.
- **C.**  
**Analysis:** To the filtrate obtained in *Identification test A* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline, shake by mechanical means for 10 min, and add 5 mL of 3 N hydrochloric acid to acidify. Chill, collect the precipitated disulfonamide of ethylenediamine, and wash with water. Recrystallize the washed precipitate from water, and dry at 105° for 1 h.  
**Acceptance criteria:** The dried precipitate melts at 164°–171°.

### ASSAY

#### • PROCEDURE

**Sample stock solution:** Transfer NLT 5 Suppositories to a tared small dish and a glass rod, and heat on a steam bath until the suppositories are melted. Mix the melt by stirring it with the rod, and cool while stirring. Transfer a portion of the cooled melt, equivalent to 1 g of aminophylline, into a beaker, add 60 mL of hot water and 3 mL of nitric acid, and heat on a steam bath for 15 min with frequent stirring. Cool, transfer to a separator with the aid of 40 mL of ether, shake well, and allow to separate, using a few mL of alcohol if necessary to bring about separation of any emulsion that has formed. Draw the water layer into a 100-mL volumetric flask; wash the ether with two 15-mL portions of water, adding the washings to the volumetric flask; and dilute with water to volume.

**Sample solution:** Transfer a portion of the *Sample stock solution*, equivalent to 250 mg of aminophylline, to a 250-mL conical flask. Add 10 mL of 6 N ammonium hydroxide and 20 mL of 0.1 N silver nitrate VS, and heat on a steam bath for 15 min. Cool to between 5° and 10° for 20 min; filter, preferably through a filtering crucible of fine porosity under reduced pressure; and wash the precipitate with small portions of water until the last washing gives NMT a faint opalescence with hydro-

chloric acid. Dissolve the precipitate by pouring over it small volumes of warm 2 N nitric acid, collecting the solution in a conical flask. Wash the filtering crucible a few times with warm water acidified with nitric acid, receiving the washings in the same flask. Cool, and add 2 mL of ferric ammonium sulfate TS.

**Analysis:** Titrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N ammonium thiocyanate is equivalent to 18.02 mg of theophylline ( $C_7H_8N_4O_2$ ).

**Acceptance criteria:** 90.0%–110.0%

### OTHER COMPONENTS

#### • CONTENT OF ETHYLENEDIAMINE

**Sample solution:** Weigh a portion of the stirred, congealed mass of the Suppositories from the *Assay*, equivalent to 500 mg of aminophylline, and place in a 500-mL conical flask. Add 150 mL of a mixture of equal volumes of alcohol and ether, and warm gently under reflux for 30 min, with occasional swirling. Cool to room temperature.

#### Titrimetric system

**Mode:** Direct titration

**Titrant:** 0.1 N hydrochloric acid VS

**Endpoint detection:** Potentiometric

**Analysis:** Titrate the *Sample solution* using a glass-modified calomel electrode system (replace the saturated potassium chloride solution of the calomel electrode with methanol saturated with lithium chloride). Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine ( $C_2H_8N_2$ ).

**Acceptance criteria:** 152–190 mg of ethylenediamine ( $C_2H_8N_2$ ) per g of theophylline ( $C_7H_8N_4O_2$ ) found in the *Assay*

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, in a cold place.
- **LABELING:** Label the Suppositories to state the content of anhydrous theophylline.

## Aminophylline Tablets

### DEFINITION

Aminophylline Tablets contain an amount of aminophylline equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of anhydrous theophylline ( $C_7H_8N_4O_2$ ).

[NOTE—The ammoniacal odor present in the vapor space above the Tablets is often quite strong, especially when bottles having suitably tight closures are newly opened. This is due to ethylenediamine vapor pressure build-up, a natural condition in the case of aminophylline.]

### IDENTIFICATION

#### Change to read:

#### • A. $\Delta$ INFRARED ABSORPTION (197K) $\Delta$ USP40

**Analysis:** Macerate a quantity of Tablets, equivalent to 500 mg of aminophylline, with 25 mL of water, and filter. The filtrate is alkaline to litmus. To the filtrate add 1 mL of 3 N hydrochloric acid, stir, and if necessary, chill to precipitate the theophylline. Filter, and retain the filtrate, free from washings.  $\Delta$ Use the filtrate in *Identification C*. $\Delta$ USP40 Wash the theophylline crystals so obtained with small quantities of ice-cold water, and dry at 105° for 1 h. $\Delta$ USP40

**Acceptance criteria:**  $\Delta$ The IR spectrum of theophylline so obtained corresponds to that of USP Theophylline RS. $\Delta$ USP40



**Change to read:**

- **B.** <sup>▲</sup>The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. <sup>▲USP40</sup>
- **C.** **Sample:** The filtrate obtained in *Identification A*  
**Analysis:** To the *Sample* add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline, shake by mechanical means for 10 min, and add 5 mL of 3 N hydrochloric acid to acidify. Chill, collect the precipitated disulfonamide of ethylenediamine, and wash with water. Recrystallize the washed precipitate from water, and dry at 105° for 1 h.  
**Acceptance criteria:** The dried precipitate melts at 164°–171°.

**ASSAY****Change to read:**• **PROCEDURE**

**Solution A:** 10 mM ammonium acetate prepared as follows. Transfer 770.8 mg of ammonium acetate to a 1-L volumetric flask, and dissolve in water to 80% of the flask volume. Adjust with glacial acetic acid to a pH of 5.5 and dilute with water to volume. Pass through a suitable filter of 0.2-μm pore size.

**Solution B:** Methanol

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	98	2
7	50	50
7.3	10	90
8.3	10	90
8.31	98	2
12	98	2

**Impurity stock solution:** 25 μg/mL of USP Theophylline Related Compound F RS in water

**System suitability solution:** 0.8 mg/mL of USP Theophylline RS and 1 μg/mL of USP Theophylline Related Compound F RS in water prepared as follows. Transfer 21 mg of USP Theophylline RS to a 25-mL volumetric flask and add 15 mL of water. Sonicate to dissolve, add 1 mL of *Impurity stock solution*, and dilute with water to volume.

**Standard solution:** 0.17 mg/mL of USP Theophylline RS in water. Sonicate to dissolve as needed.

**Sample solution:** Nominally 0.17 mg/mL of anhydrous theophylline from NLT 20 finely powdered Tablets in water prepared as follows. Transfer 34 mg of anhydrous theophylline from a portion of the powder to a 200-mL volumetric flask. Add 20 mL of water and mix for 1 min. Add an additional 140 mL of water and sonicate for 30 min. Dilute with water to volume. Pass through a suitable filter of 0.22-μm pore size, discarding the first 2–3 mL.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 2.1-mm × 10-cm; 1.7-μm packing L1

**Column temperature:** 40°

**Flow rate:** 0.4 mL/min

**Injection volume:** 1 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of theophylline from the *Sample solution*

$r_S$  = peak response of theophylline from the *Standard solution*

$C_S$  = concentration of USP Theophylline RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of theophylline in the *Sample solution* (mg/mL) <sup>▲USP40</sup>

**Acceptance criteria:** 93.0%–107.0%

**OTHER COMPONENTS**• **CONTENT OF ETHYLENEDIAMINE**

**Sample solution:** Transfer a portion of the powdered Tablets, equivalent to 350 mg of aminophylline, prepared in the *Assay*, into a 100-mL conical flask. Add 20 mL of water, and digest at 50°, with frequent shaking, for 30 min. Cool, filter into a 250-mL conical flask, and wash with water until the last washing is neutral to litmus. Use the combined filtrate and washings.

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N hydrochloric acid VS

**Endpoint detection:** Visual

**Analysis:** Add methyl orange TS to the *Sample solution*, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>).

**Acceptance criteria:** 140–190 mg of ethylenediamine (C<sub>2</sub>H<sub>8</sub>N<sub>2</sub>) per gram of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) found in the *Assay*

**PERFORMANCE TESTS**• **DISSOLUTION (711)**

**For uncoated or plain coated tablets**

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Standard solution:** USP Theophylline RS in *Medium*

**Sample solution:** Proceed as directed in the chapter for sample. Dilute with water to a concentration that is similar to that of the *Standard solution*.

**Instrumental conditions**

**Mode:** UV-Vis

**Analytical wavelength:** UV about 269 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) dissolved.

**Tolerances:** NLT 75% (Q) of the labeled amount of theophylline (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905)****Procedure for content uniformity**

**Standard solution:** 10 μg/mL of USP Theophylline RS

**Sample solution:** Place 1 Tablet in a 250-mL volumetric flask, add 200 mL of water, and shake by mechanical means until disintegration is complete. Add water to volume. Filter a portion of the mixture, discarding the first 20 mL of the filtrate.



**Instrumental conditions**

Mode: UV

Analytical wavelength: About 269 nm

Cell: 1 cm

Blank: Water

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of anhydrous theophylline ( $C_7H_8N_4O_2$ ) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

 $A_U$  = absorbance of the *Sample solution* $A_S$  = absorbance of the *Standard solution* $C_S$  = concentration of USP Theophylline RS in the *Standard solution* ( $\mu\text{g/mL}$ ) $C_U$  = nominal concentration of theophylline in the *Sample solution* ( $\text{mg/mL}$ )

Acceptance criteria: Meet the requirements

**IMPURITIES****Add the following:****▲ ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Impurity stock solution, System suitability solution, and Chromatographic system: Proceed as directed in the Assay.

**Standard solution:** 2.0  $\mu\text{g/mL}$  each of USP Theophylline RS and USP Theophylline Related Compound D RS in water**Sample solution:** Nominally 1.0  $\text{mg/mL}$  of anhydrous aminophylline from NLT 20 finely powdered Tablets in water prepared as follows. Transfer 10  $\text{mg}$  of anhydrous aminophylline from a portion of the powder to a 10-mL volumetric flask. Add 5 mL of water and sonicate for 30 min. Dilute with water to volume. Pass through a suitable filter of 0.22- $\mu\text{m}$  pore size, discarding the first 2–3 mL.**System suitability**Samples: *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for relative retention times.]

**Suitability requirements****Resolution:** NLT 2.0 between theophylline and theophylline related compound F, *System suitability solution***Relative standard deviation:** NMT 3.0% for each peak, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of theophylline related compound D in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of theophylline related compound D from the *Sample solution* $r_S$  = peak response of theophylline related compound D from the *Standard solution* $C_S$  = concentration of USP Theophylline Related Compound D RS in the *Standard solution* ( $\text{mg/mL}$ ) $C_U$  = nominal concentration of aminophylline in the *Sample solution* ( $\text{mg/mL}$ )

Calculate the percentage of any other individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of any other individual unspecified degradation product from the *Sample solution* $r_S$  = peak response of theophylline from the *Standard solution* $C_S$  = concentration of USP Theophylline RS in the *Standard solution* ( $\text{mg/mL}$ ) $C_U$  = nominal concentration of aminophylline in the *Sample solution* ( $\text{mg/mL}$ )

Acceptance criteria: See Table 2. Disregard peaks less than 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Theophylline related compound C <sup>a,b</sup>	0.36	—
Theophylline related compound B <sup>a,c</sup>	0.63	—
Theophylline related compound D	0.69	0.2
Dimethyl uric acid <sup>a,d</sup>	0.76	—
Theobromine <sup>a,e</sup>	0.82	—
Theophylline	1.0	—
Theophylline related compound F <sup>a</sup>	1.09	—
Caffeine <sup>a</sup>	1.20	—
Any other individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

<sup>a</sup> Process impurity included for identification only and not to be included in the calculation of total degradation products.<sup>b</sup> N-(6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)formamide.<sup>c</sup> 3-Methyl-1H-purine-2,6-dione.<sup>d</sup> 1,3-Dimethyl-7,9-dihydro-1H-purine-2,6,8(3H)-trione.<sup>e</sup> 3,7-Dihydro-3,7-dimethylpurine-2,6(1H)-dione.

▲ USP40

**ADDITIONAL REQUIREMENTS****Change to read:**

- **PACKAGING AND STORAGE:** Preserve in tight containers.  
▲Store at controlled room temperature.▲USP40
- **LABELING:** Label the Tablets to state the content of anhydrous theophylline.

**Change to read:**• **USP REFERENCE STANDARDS (11)**

USP Theophylline RS

▲USP Theophylline Related Compound D RS

N-Methyl-5-(methylamino)-1H-imidazole-4-carboxamide.

 $C_6H_{10}N_4O$  154.17

USP Theophylline Related Compound F RS

7-(2-Hydroxyethyl)-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione.

 $C_9H_{12}N_4O_3$  224.22▲USP40**Aminophylline Delayed-Release Tablets****DEFINITION**Aminophylline Delayed-Release Tablets contain an amount of aminophylline equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of anhydrous theophylline ( $C_7H_8N_4O_2$ ).

[NOTE—The ammoniacal odor present in the vapor space above Tablets is often quite strong, especially when bottles having suitably tight closures are newly opened. This is due to ethylenediamine vapor pressure build-up, a natural condition in the case of aminophylline.]



**IDENTIFICATION**

- **A.**  
**Analysis:** Macerate a quantity of Tablets, equivalent to 500 mg of aminophylline, with 25 mL of water, and filter: the filtrate is alkaline to litmus. To the filtrate add 1 mL of 3 N hydrochloric acid, stir, and if necessary, chill to precipitate the theophylline. Filter, and retain the filtrate, free from washings. Wash the theophylline crystals so obtained with small quantities of ice-cold water, and dry at 105° for 1 h. Transfer 10 mg of the dried theophylline crystals to a porcelain dish, add 1 mL of hydrochloric acid and 100 mg of potassium chlorate, evaporate on a steam bath to dryness, and invert the dish over a vessel containing a few drops of 6 N ammonium hydroxide.  
**Acceptance criteria:** The residue acquires a purple color, which is destroyed by solutions of fixed alkalis.
- **B.**  
**Analysis:** Recrystallize the dried theophylline crystals from *Identification* test A from water, and dry at 105° for 1 h.  
**Acceptance criteria:** The recrystallized theophylline melts at 270°–274°.
- **C.**  
**Analysis:** To the filtrate obtained in *Identification* test A add 0.5 mL of benzenesulfonyl chloride and 5 mL of 1 N sodium hydroxide to render alkaline, shake by mechanical means for 10 min, and add 5 mL of 3 N hydrochloric acid to acidify. Chill, collect the precipitated disulfonamide of ethylenediamine, and wash with water. Recrystallize the washed precipitate from water, and dry at 105° for 1 h.  
**Acceptance criteria:** The dried precipitate melts at 164°–171°.

**ASSAY**• **PROCEDURE**

**Sample solution:** Transfer an equivalent to 2 g of aminophylline, from powdered Tablets (NLT 20), to a 200-mL volumetric flask with the aid of a mixture of 50 mL of water and 15 mL of 6 N ammonium hydroxide, and allow to stand for 30 min with frequent shaking, warming to 50° if necessary to dissolve the aminophylline. Cool the mixture to room temperature if it has been warmed, and add water to volume. Centrifuge 50 mL of the mixture; pipet a portion of the clear supernatant, equivalent to 250 mg of aminophylline, into a 250-mL conical flask; and dilute with water if necessary to make 40 mL. Add 8 mL of 6 N ammonium hydroxide and 20.0 mL of 0.1 N silver nitrate VS, mix, heat to boiling, and continue boiling for 15 min. Cool to between 5° and 10° for 20 min, then filter, preferably through a filtering crucible under reduced pressure, and wash the precipitate with three 10-mL portions of water. Acidify the combined filtrate and washings with nitric acid, and add an additional 3 mL of the acid. Cool, and add 2 mL of ferric ammonium sulfate TS.

**Titrimetric system**

**Mode:** Residual titration

**Titrant:** 0.1 N ammonium thiocyanate VS

**Endpoint detection:** Visual

**Analysis:** Titrate the excess silver nitrate with *Titrant*. Each mL of 0.1 N silver nitrate is equivalent to 18.02 mg of theophylline ( $C_7H_8N_4O_2$ ).

**Acceptance criteria:** 93.0%–107.0%

**OTHER COMPONENTS**• **CONTENT OF ETHYLENEDIAMINE**

**Sample solution:** Transfer a portion, equivalent to 350 mg of aminophylline, of the powdered Tablets prepared in the Assay into a 100-mL conical flask. Add 20 mL of water, and digest at 50°, with frequent shaking, for 30 min. Cool, filter into a 250-mL conical flask, and wash with water until the last washing is neutral to litmus. Use the combined filtrate and washings.

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N hydrochloric acid VS

**Endpoint detection:** Visual

**Analysis:** Add methyl orange TS to the *Sample solution*, and titrate. Each mL of 0.1 N hydrochloric acid is equivalent to 3.005 mg of ethylenediamine ( $C_2H_8N_2$ ).

**Acceptance criteria:** 140–190 mg of ethylenediamine ( $C_2H_8N_2$ ) per g of theophylline ( $C_7H_8N_4O_2$ ) found in the Assay

**PERFORMANCE TESTS**

- **DISINTEGRATION (701):** 30 min, determined as directed in *Delayed-Release (Enteric-Coated) Tablets*

- **UNIFORMITY OF DOSAGE UNITS (905)**

**Procedure for content uniformity**

**Standard solution:** 10 µg/mL of USP Theophylline RS

**Sample solution:** Place 1 Tablet in a 250-mL volumetric flask, add 200 mL of water, and shake by mechanical means until disintegration is complete. Add water to volume. Filter a portion of the mixture, discarding the first 20 mL of the filtrate.

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** About 269 nm

**Cell:** 1 cm

**Blank:** Water

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of theophylline ( $C_7H_8N_4O_2$ ) in each Tablet:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Theophylline RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of theophylline in the *Sample solution* (µg/mL)

**Acceptance criteria:** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label the Tablets to state the content of anhydrous theophylline.
- **USP REFERENCE STANDARDS (11)**  
 USP Theophylline RS

**Aminosaliclylate Sodium**

$C_7H_6NNaO_3 \cdot 2H_2O$	211.15
$C_7H_6NNaO_3$	175.12
Benzoic acid, 4-amino-2-hydroxy-, monosodium salt, dihydrate;	
Monosodium 4-aminosalicylate dihydrate [6018-19-5].	
Anhydrous [133-10-8].	

**DEFINITION**

Aminosaliclylate Sodium contains NLT 98.0% and NMT 101.0% of aminosaliclylate sodium ( $C_7H_6NNaO_3$ ), calculated on the anhydrous basis.

**[CAUTION]**—Prepare solutions of Aminosaliclylate Sodium within 24 h of administration. Under no circumstances use a solution if its color is darker than that of a freshly prepared solution.]

**IDENTIFICATION**• **A.**

**Sample stock solution:** Dissolve 250 mg in 3 mL of 1 N sodium hydroxide, transfer to a 500-mL volumetric flask, and dilute with water to volume.



**Sample solution:** Transfer a 5-mL aliquot of the *Sample stock solution* to a 250-mL volumetric flask containing 12.5 mL of pH 7 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*), and dilute with water to volume.

**Analysis:** Compare the *Sample solution* in a suitable spectrometer against a blank of the same buffer in the same concentration.

**Acceptance criteria:** The *Sample solution* exhibits absorbance maxima at  $265 \pm 2$  and  $299 \pm 2$  nm, and the ratio  $A_{265}/A_{299}$  is 1.50–1.56.

• **B.**

**Sample:** 1 g

**Analysis:** Place the *Sample* into a small, round-bottom flask, and add 10 mL of acetic anhydride. Heat the flask on a steam bath for 30 min, add 40 mL of water, filter, cool, and allow to stand until the diacetyl derivative has crystallized. Collect the precipitate on a filter, wash well with water, and dry at  $105^\circ$  for 1 h.

**Acceptance criteria:** The diacetyl derivative melts at  $191^\circ$ – $197^\circ$ .

• **C.**

**Sample:** 50 mg

**Analysis:** Dissolve the *Sample* in 5 mL of water, add 1 mL of 3 N hydrochloric acid, and filter if necessary. To the filtrate add 1 drop of ferric chloride TS.

**Acceptance criteria:** A violet color is produced.

• **D. IDENTIFICATIONS TESTS—GENERAL, Sodium (191):** Meets the requirements

**ASSAY**

• **PROCEDURE**

**Solution A:** 12.7 mg/mL of tetrabutylammonium hydroxide in methanol

**Mobile phase:** *Solution A*, 0.05 M dibasic sodium phosphate, and 0.05 M monobasic sodium phosphate (150:425:425)

**Internal standard solution:** 5 mg/mL of acetaminophen in *Mobile phase*

**Standard solution:** 0.5 mg/mL of aminosaliclylic acid prepared as follows. Transfer 12.5 mg of USP Aminosaliclylic Acid RS to a 25-mL low-actinic volumetric flask, add 15 mL of *Mobile phase*, and swirl to dissolve. Add 2.5 mL of *Internal standard solution*, and dilute with *Mobile phase* to volume.

**Sample solution:** 0.69 mg/mL of Aminosaliclylate Sodium prepared as follows. Transfer 69 mg of Aminosaliclylic Acid to a 100-mL low-actinic volumetric flask, add 50 mL of *Mobile phase*, and swirl to dissolve. Add 10.0 mL of *Internal standard solution*, and dilute with *Mobile phase* to volume.

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for acetaminophen and aminosaliclylic acid are 0.83 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.7 between aminosaliclylic acid and acetaminophen

**Relative standard deviation:** NMT 1.0% for the peak response ratio of aminosaliclylic acid to acetaminophen

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

After use, wash the column for 30 min with methanol, water, and phosphoric acid (77: 23: 0.6), and then wash for 30 min with methanol and water (50:50).

Calculate the percentage of aminosaliclylate sodium ( $C_7H_6NNaO_3$ ) in the sample taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak response ratio of aminosaliclylate to acetaminophen from the *Sample solution*

$R_S$  = peak response ratio of aminosaliclylic acid to acetaminophen from the *Standard solution*

$C_S$  = concentration of USP Aminosaliclylic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aminosaliclylate Sodium in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of anhydrous aminosaliclylate sodium, 175.12

$M_{r2}$  = molecular weight of aminosaliclylic acid, 153.14

**Acceptance criteria:** 98.0%–101.0% on the anhydrous basis

**IMPURITIES**

• **CHLORIDE AND SULFATE, Chloride (221)**

**Sample solution:** 25 mg/mL in a mixture of 5 mL of nitric acid and 15 mL of water

**Acceptance criteria:** NMT 0.042%; the solution shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid.

**Delete the following:**

• **HEAVY METALS, Method II (231):** NMT 30 ppm (Official 1: Jan-2018)

• **LIMIT OF *m*-AMINOPHENOL**

**Solution A:** 12.7 mg/mL of tetrabutylammonium hydroxide in methanol

**Mobile phase:** *Solution A*, 0.05 M dibasic sodium phosphate, and 0.05 M monobasic sodium phosphate (150:425:425)

**Internal standard solution:** 5  $\mu$ g/mL of sulfanilamide in *Mobile phase*

**Standard stock solution:** 12  $\mu$ g/mL of USP *m*-Aminophenol RS in *Mobile phase*

**Standard solution:** 1.2  $\mu$ g/mL of USP *m*-Aminophenol RS prepared as follows. Transfer 10.0 mL of the *Standard stock solution* and 10.0 mL of the *Internal standard solution* to a 100-mL low-actinic volumetric flask, and dilute with *Mobile phase* to volume.

**Sample solution:** Use the *Sample solution* prepared as directed in the Assay, except use 10.0 mL of sulfanilamide as the *Internal standard solution*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  25-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for sulfanilamide and *m*-aminophenol are 0.66 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.5 between *m*-aminophenol and sulfanilamide

**Relative standard deviation:** NMT 7%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

After use, wash the column for 30 min with methanol, water, and phosphoric acid (77: 23: 0.6), and then wash for 30 min with methanol and water (50:50).

Calculate the percentage of *m*-aminophenol in the portion of Aminosaliclylate Sodium taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$



- $R_U$  = peak response ratio of *m*-aminophenol to sulfanilamide from the *Sample solution*  
 $R_S$  = peak response ratio of *m*-aminophenol to sulfanilamide from the *Standard solution*  
 $C_S$  = concentration of USP *m*-Aminophenol RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of the *Sample solution* (mg/mL), as determined in the *Assay*  
 Acceptance criteria: NMT 0.25%

### SPECIFIC TESTS

#### • PH (791)

Sample solution: 20 mg/mL  
 Acceptance criteria: 6.5–8.5

#### • WATER DETERMINATION, Method I (921): 16.0%–18.0%

#### • HYDROGEN SULFIDE, SULFUR DIOXIDE, AND AMYL ALCOHOL

Sample: 500 mg

Analysis: Dissolve the *Sample* in 5 mL of 1 N sodium hydroxide, add 6 mL of 3 N hydrochloric acid, and stir vigorously.

Acceptance criteria: No odor of hydrogen sulfide or sulfur dioxide is perceptible, and NMT a faint odor of amyl alcohol is perceptible. A piece of moistened lead acetate test paper held over the mixture does not become discolored.

#### • CLARITY AND COLOR OF SOLUTION

Sample 1: 1 g

Analysis 1: Dissolve *Sample 1* in 10 mL of water.

Acceptance criteria 1: The resulting solution is clear and has NMT a faint yellow color.

Sample 2: 1 g

Analysis 2: Dissolve *Sample 2* in 50 mL of a freshly prepared mixture of 5 mL of nitric acid and 45 mL of water.

Acceptance criteria 2: The resulting solution is clear and has NMT a slight color.

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in tight, light-resistant containers, protected from excessive heat.

#### • USP REFERENCE STANDARDS (11)

USP *m*-Aminophenol RS  
 USP Aminosaliclylic Acid RS

## Aminosaliclylate Sodium Tablets

### DEFINITION

Aminosaliclylate Sodium Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of aminosaliclylate sodium ( $C_7H_6NNaO_3 \cdot 2H_2O$ ).

### IDENTIFICATION

**Sample:** Mix powdered Tablets, equivalent to 3 g of aminosaliclylate sodium, with 40 mL of water, and filter. Add to the filtrate 15 mL of 1 N acetic acid, and allow to stand until precipitation has occurred. Collect the precipitate on a filter, wash well with water, and dry at 105° for 30 min.

#### • A.

Sample: 1 g

Analysis: Place the *Sample* in a small, round-bottom flask, and add 10 mL of acetic anhydride. Heat the flask on a steam bath for 30 min, add 40 mL of water, filter, cool, and allow to stand until the diacetyl derivative has crystallized. Collect the precipitate on a filter, wash well with water, and dry at 105° for 1 h.

Acceptance criteria: The diacetyl derivative melts at 191°–197°.

#### • B.

Sample: 0.1 g

Analysis: Shake the *Sample* with 10 mL of water, and filter. To 5 mL of the filtrate add 1 drop of ferric chloride TS.

Acceptance criteria: A violet color is produced.

### ASSAY

#### • PROCEDURE

**Solution A:** 12.7 mg/mL of tetrabutylammonium hydroxide in methanol

**Mobile phase:** *Solution A*, 0.05 M dibasic sodium phosphate, and 0.05 M monobasic sodium phosphate (150:425:425)

**Internal standard solution:** 5 mg/mL of acetaminophen in *Mobile phase*

**Standard solution:** 0.5 mg/mL of aminosaliclylic acid prepared as follows. Transfer 12.5 mg of USP Aminosaliclylic Acid RS to a 25-mL low-actinic volumetric flask, add 15 mL of *Mobile phase*, and swirl to dissolve. Add 2.5 mL of *Internal standard solution*, and dilute with *Mobile phase* to volume.

**Sample stock solution:** 6.9 mg/mL of aminosaliclylate sodium in *Mobile phase* prepared as follows. Add nominally 690 mg of aminosaliclylate sodium from NLT 20 powdered Tablets to a 100-mL low-actinic volumetric flask. Add 50 mL of *Mobile phase*, and shake for 5 min. Dilute with *Mobile phase* to volume, and filter.

**Sample solution:** 0.69 mg/mL of aminosaliclylate from the *Sample stock solution* prepared as follows. Transfer 10.0 mL of the clear filtrate to a 100-mL low-actinic volumetric flask containing 10.0 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for acetaminophen and aminosaliclylic acid are 0.83 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.7 between aminosaliclylic acid and acetaminophen

**Relative standard deviation:** NMT 1.0% for the peak response ratio of aminosaliclylic acid to acetaminophen

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

After use, wash the column for 30 min with a mixture of methanol, water, and phosphoric acid (77: 23: 0.6), and then wash for 30 min with a mixture of methanol and water (50:50).

Calculate the percentage of the labeled amount of aminosaliclylate sodium ( $C_7H_6NNaO_3 \cdot 2H_2O$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak response ratio of aminosaliclylate to acetaminophen from the *Sample solution*

$R_S$  = peak response ratio of aminosaliclylic acid to acetaminophen from the *Standard solution*

$C_S$  = concentration of USP Aminosaliclylic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aminosaliclylate sodium in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of aminosaliclylate sodium dihydrate, 211.15

$M_{r2}$  = molecular weight of aminosaliclylic acid, 153.14



Acceptance criteria: 95.0%–105.0%

## PERFORMANCE TESTS

- **DISSOLUTION**, *Procedure for a Pooled Sample* (711)  
Medium: Water; 900 mL  
Apparatus 1: 100 rpm  
Time: 45 min  
Analysis: Determine the percentage of the labeled amount of aminosaliclylate sodium ( $C_7H_6NNaO_3 \cdot 2H_2O$ ) dissolved, using the procedure as directed in the *Assay*, making any necessary modifications.  
Tolerances: NLT 75% (Q) of the labeled amount of aminosaliclylate sodium ( $C_7H_6NNaO_3 \cdot 2H_2O$ ) is dissolved.
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

## IMPURITIES

### LIMIT OF *m*-AMINOPHENOL

- Solution A:** 12.7 mg/mL of tetrabutylammonium hydroxide in methanol
- Mobile phase:** *Solution A*, 0.05 M dibasic sodium phosphate, and 0.05 M monobasic sodium phosphate (150:425:425)
- Internal standard solution:** 5 µg/mL of sulfanilamide in *Mobile phase*
- Standard stock solution:** 12 µg/mL of USP *m*-Aminophenol RS in *Mobile phase*
- Standard solution:** 1.2 µg/mL of USP *m*-Aminophenol RS prepared as follows. Transfer 10.0 mL of the *Standard stock solution* and 10.0 mL of the *Internal standard solution* to a 100-mL low-actinic volumetric flask, and dilute with *Mobile phase* to volume.
- Sample solution:** Use the *Sample solution* prepared as directed in the *Assay*, except use 10.0 mL of sulfanilamide as the *Internal standard solution*.

### Chromatographic system

- (See *Chromatography* (621), *System Suitability*.)
- Mode:** LC
- Detector:** UV 280 nm
- Column:** 4.6-mm × 25-cm; 10-µm packing L1
- Flow rate:** 1.5 mL/min
- Injection volume:** 20 µL

### System suitability

- Sample:** *Standard solution*  
[NOTE—The relative retention times for sulfanilamide and *m*-aminophenol are 0.66 and 1.0, respectively.]
- Suitability requirements**  
**Resolution:** NLT 2.5 between *m*-aminophenol and sulfanilamide
- Relative standard deviation:** NMT 7%

### Analysis

- Samples:** *Standard solution* and *Sample solution*  
After use, wash the column for 30 min with a mixture of methanol, water, and phosphoric acid (77: 23: 0.6), and then wash for 30 min with a mixture of methanol and water (50:50).  
Calculate the percentage of *m*-aminophenol in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

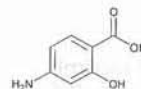
- $R_U$  = peak response ratio of *m*-aminophenol to sulfanilamide from the *Sample solution*
- $R_S$  = peak response ratio of *m*-aminophenol to sulfanilamide from the *Standard solution*
- $C_S$  = concentration of USP *m*-Aminophenol RS in the *Standard solution* (mg/mL)
- $C_U$  = nominal concentration of aminosaliclylate sodium in the *Sample solution*, as determined in the *Assay* (mg/mL)

Acceptance criteria: NMT 1.0%

## USP REFERENCE STANDARDS (11)

- USP *m*-Aminophenol RS
- USP Aminosaliclylic Acid RS

## Aminosaliclylic Acid



$C_7H_7NO_3$  153.14  
Benzoic acid, 4-amino-2-hydroxy-;  
4-Aminosaliclylic acid [65-49-6].

## DEFINITION

Aminosaliclylic Acid contains NLT 98.5% and NMT 100.5% of aminosaliclylic acid ( $C_7H_7NO_3$ ), calculated on the anhydrous basis.

[CAUTION—Under no circumstances use a solution prepared from Aminosaliclylic Acid if its color is darker than that of a freshly prepared solution.]

## IDENTIFICATION

- **A.**  
**Sample stock solution:** Dissolve 250 mg in 3 mL of 1 N sodium hydroxide, transfer to a 500-mL volumetric flask, and dilute with water to volume.  
**Sample solution:** Transfer a 5-mL aliquot of the *Sample stock solution* to a 250-mL volumetric flask containing 12.5 mL of pH 7 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*), and dilute with water to volume.  
**Analysis:** Compare the *Sample solution* in a suitable spectrometer against a blank of the same buffer in the same concentration.  
**Acceptance criteria:** The *Sample solution* exhibits absorbance maxima at  $265 \pm 2$  and  $299 \pm 2$  nm, and the ratio  $A_{265}/A_{299}$  is 1.50–1.56.
- **B.**  
**Sample:** 1 g  
**Analysis:** Place the *Sample* in a small, round-bottom flask, and add 10 mL of acetic anhydride. Heat the flask on a steam bath for 30 min, add 40 mL of water, filter, cool, and allow to stand until the diacetyl derivative has crystallized. Collect the precipitate on a filter, wash well with water, and dry at  $105^\circ$  for 1 h.  
**Acceptance criteria:** The diacetyl derivative melts at  $191^\circ$ – $197^\circ$ .
- **C.**  
**Sample:** 0.1 g  
**Analysis:** Shake the *Sample* with 10 mL of water, and filter. To 5 mL of the filtrate add 1 drop of ferric chloride TS.  
**Acceptance criteria:** A violet color is produced.

## ASSAY

### PROCEDURE

- Solution A:** 12.7 mg/mL of tetrabutylammonium hydroxide in methanol
- Mobile phase:** *Solution A*, 0.05 M dibasic sodium phosphate, and 0.05 M monobasic sodium phosphate (150:425:425)
- Internal standard solution:** 5 mg/mL of acetaminophen in *Mobile phase*
- Standard solution:** 0.5 mg/mL of aminosaliclylic acid prepared as follows. Transfer 12.5 mg of USP Aminosaliclylic Acid RS to a 25-mL low-actinic volumetric flask, add 15 mL of *Mobile phase*, and swirl to dissolve. Add 2.5 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.



**Sample solution:** 0.5 mg/mL of Aminosalicyclic Acid prepared as follows. Transfer 12.5 mg of Aminosalicyclic Acid to a 25-mL low-actinic volumetric flask, add 15 mL of *Mobile phase*, and swirl to dissolve. Add 2.5 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for acetaminophen and aminosalicyclic acid are 0.83 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.7 between aminosalicyclic acid and acetaminophen

**Relative standard deviation:** NMT 1.0% for the peak response ratio of aminosalicyclic acid to acetaminophen

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

After use, wash the column for 30 min with a mixture of methanol, water, and phosphoric acid (77: 23: 0.6), and then wash for 30 min with a mixture of methanol and water (50:50).

Calculate the percentage of aminosalicyclic acid ( $C_7H_7NO_3$ ) in the portion of Aminosalicyclic Acid taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of aminosalicyclic acid to acetaminophen from the *Sample solution*

$R_S$  = peak response ratio of aminosalicyclic acid to acetaminophen from the *Standard solution*

$C_S$  = concentration of USP Aminosalicyclic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aminosalicyclic Acid in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.5%–100.5% on the anhydrous basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

#### Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 30 ppm • (Official 1-Jan-2018)
- **CHLORIDE AND SULFATE**, *Chloride* (221)  
**Sample solution:** 25 mg/mL in a mixture of nitric acid and water (5:15)  
**Acceptance criteria:** NMT 0.042%; the solution shows no more chloride than corresponds to 0.30 mL of 0.020 N hydrochloric acid.
- **LIMIT OF *m*-AMINOPHENOL**  
**Solution A:** 12.7 mg/mL of tetrabutylammonium hydroxide in methanol  
**Mobile phase:** *Solution A*, 0.05 M dibasic sodium phosphate, and 0.05 M monobasic sodium phosphate (150:425:425)  
**Internal standard solution:** 5 µg/mL of sulfanilamide in *Mobile phase*  
**Standard stock solution:** 12 µg/mL of USP *m*-Aminophenol RS in *Mobile phase*  
**Standard solution:** 1.2 µg/mL of USP *m*-Aminophenol RS prepared as follows. Transfer 10.0 mL of the *Standard stock solution* and 10.0 mL of the *Internal standard solution* to a 100-mL low-actinic volumetric flask, and dilute with *Mobile phase* to volume.

**Sample solution:** 0.5 mg/mL of Aminosalicyclic Acid prepared as follows. Transfer 50 mg of Aminosalicyclic Acid to a 100-mL low-actinic volumetric flask, add 50 mL of *Mobile phase*, and swirl to dissolve. Add 10.0 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 10-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for sulfanilamide and *m*-aminophenol are about 0.66 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between *m*-aminophenol and sulfanilamide

**Relative standard deviation:** NMT 7%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

After use, wash the column for 30 min with a mixture of methanol, water, and phosphoric acid (77: 23: 0.6), and then wash for 30 min with a mixture of methanol and water (50:50).

Calculate the percentage of *m*-aminophenol in the portion of Aminosalicyclic Acid taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of *m*-aminophenol to sulfanilamide from the *Sample solution*

$R_S$  = peak response ratio of *m*-aminophenol to sulfanilamide from the *Standard solution*

$C_S$  = concentration of USP *m*-Aminophenol RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution*, as determined in the *Assay* (mg/mL)

**Acceptance criteria:** NMT 0.25%

#### SPECIFIC TESTS

- **pH** (791): 3.0–3.7, in a saturated solution
- **WATER DETERMINATION**, *Method I* (921): NMT 0.5%
- **HYDROGEN SULFIDE, SULFUR DIOXIDE, AND AMYL ALCOHOL**  
**Sample:** 500 mg  
**Analysis:** Dissolve the *Sample* in 5 mL of 1 N sodium hydroxide, add 6 mL of 3 N hydrochloric acid, and stir vigorously.

**Acceptance criteria:** No odor of hydrogen sulfide or sulfur dioxide is perceptible, and NMT a faint odor of amyl alcohol is perceptible. A piece of moistened lead acetate test paper held over the mixture does not become discolored.

#### CLARITY AND COLOR OF SOLUTION

**Sample 1:** 1 g

**Analysis 1:** Dissolve *Sample 1* in 10 mL of sodium bicarbonate solution (1 in 15).

**Acceptance criteria 1:** The resulting solution is clear and has NMT a faint yellow color.

**Sample 2:** 1 g

**Analysis 2:** Dissolve *Sample 2* in 50 mL of freshly prepared 1.6 M nitric acid.

**Acceptance criteria 2:** The resulting solution is clear and has NMT a slight color.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature not exceeding 30°.



• **USP REFERENCE STANDARDS (11)**

USP *m*-Aminophenol RS

USP Aminosalicyclic Acid RS

## Aminosalicyclic Acid Tablets

### DEFINITION

Aminosalicyclic Acid Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of aminosalicyclic acid ( $C_7H_7NO_3$ ).

### IDENTIFICATION

**Sample:** Mix powdered Tablets, equivalent to 2 g of aminosalicyclic acid, with 50 mL of a mixture of acetone and chloroform (1:2), and filter. Evaporate the filtrate with a current of warm air to dryness.

• **A.**

**Sample:** 1 g

**Analysis:** Place the *Sample* in a small, round-bottom flask, and add 10 mL of acetic anhydride. Heat the flask on a steam bath for 30 min, add 40 mL of water, filter, cool, and allow to stand until the diacetyl derivative has crystallized. Collect the precipitate on a filter, wash well with water, and dry at 105° for 1 h.

**Acceptance criteria:** The diacetyl derivative melts at 191°–197°.

• **B.**

**Sample:** 0.1 g

**Analysis:** Shake the *Sample* with 10 mL of water, and filter. To 5 mL of the filtrate add 1 drop of ferric chloride TS.

**Acceptance criteria:** A violet color is produced.

### ASSAY

• **PROCEDURE**

**Solution A:** 12.7 mg/mL of tetrabutylammonium hydroxide in methanol

**Mobile phase:** *Solution A*, 0.05 M dibasic sodium phosphate, and 0.05 M monobasic sodium phosphate (150:425:425)

**Internal standard solution:** 5 mg/mL of acetaminophen in *Mobile phase*

**Standard solution:** 0.5 mg/mL of aminosalicyclic acid prepared as follows. Transfer 12.5 mg of USP Aminosalicyclic Acid RS to a 25-mL low-actinic volumetric flask, add 15 mL of *Mobile phase*, and swirl to dissolve. Add 2.5 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.

**Sample stock solution:** Add nominally 500 mg of aminosalicyclic acid from NLT 20 powdered Tablets to a 100-mL low-actinic volumetric flask. Add 50 mL of *Mobile phase*, and shake for 5 min. Dilute with *Mobile phase* to volume, and filter.

**Sample solution:** Transfer 10.0 mL of the clear filtrate from the *Sample stock solution* to a 100-mL low-actinic volumetric flask containing 10.0 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for acetaminophen and aminosalicyclic acid are 0.83 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.7 between aminosalicyclic acid and acetaminophen

**Relative standard deviation:** NMT 1.0% for the peak response ratio of aminosalicyclic acid to acetaminophen

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

After use, wash the column for 30 min with a mixture of methanol, water, and phosphoric acid (77: 23: 0.6), and then wash for 30 min with a mixture of methanol and water (50:50).

Calculate the percentage of the labeled amount of aminosalicyclic acid ( $C_7H_7NO_3$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of aminosalicyclic acid to acetaminophen from the *Sample solution*

$R_S$  = peak response ratio of aminosalicyclic acid to acetaminophen from the *Standard solution*

$C_S$  = concentration of USP Aminosalicyclic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aminosalicyclic acid in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0%

### PERFORMANCE TESTS

• **DISSOLUTION (711)**

**Medium:** pH 7.5 phosphate buffer; 900 mL (see *Reagents, Indicators, and Solutions—Buffer Solutions*)

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Analysis:** Calculate the percentage of the labeled amount of aminosalicyclic acid ( $C_7H_7NO_3$ ) dissolved, using the procedure as directed in the *Assay*, making any necessary modifications.

**Tolerances:** NLT 75% (Q) of the labeled amount of aminosalicyclic acid ( $C_7H_7NO_3$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

### IMPURITIES

• **LIMIT OF *m*-AMINOPHENOL**

**Mobile phase:** Prepare as directed in the *Assay*.

**Internal standard solution:** 5 µg/mL of sulfanilamide in *Mobile phase*

**Standard stock solution:** 12 µg/mL of USP *m*-Aminophenol RS in *Mobile phase*

**Standard solution:** *Standard stock solution*, *Internal standard solution*, and *Mobile phase* (1:1:8) in a low-actinic volumetric flask

**Sample solution:** Use the *Sample solution* prepared as directed in the *Assay*, except use 10.0 mL of sulfanilamide for the *Internal standard solution*.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 10-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for sulfanilamide and *m*-aminophenol are 0.66 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between *m*-aminophenol and sulfanilamide

**Relative standard deviation:** NMT 7%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

After use, wash the column for 30 min with a mixture of methanol, water, and phosphoric acid (77: 23: 0.6), and then wash for 30 min with a mixture of methanol and water (50:50).



Calculate the percentage of *m*-aminophenol in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

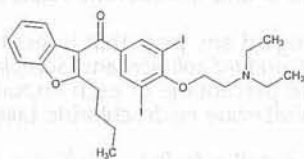
- $R_U$  = peak response ratio of *m*-aminophenol to sulfanilamide from the *Sample solution*  
 $R_S$  = peak response ratio of *m*-aminophenol to sulfanilamide from the *Standard solution*  
 $C_S$  = concentration of USP *m*-Aminophenol RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of aminosalicic acid in the *Sample solution*, as determined in the Assay (mg/mL)

Acceptance criteria: NMT 1.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature not exceeding 30°.
- **USP REFERENCE STANDARDS** (11)  
 USP *m*-Aminophenol RS  
 USP Aminosalicic Acid RS

## Amiodarone Hydrochloride



$C_{25}H_{29}I_2NO_3 \cdot HCl$  681.77  
 Methanone, (2-butyl-3-benzofuranyl)[4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]- hydrochloride; 2-Butyl-3-benzofuranyl 4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone hydrochloride [19774-82-4]. 2-Butyl-3-benzofuranyl 4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone [1951-25-3].

#### DEFINITION

Amiodarone Hydrochloride contains NLT 98.5% and NMT 101.0% of  $C_{25}H_{29}I_2NO_3 \cdot HCl$ , calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

#### ASSAY

##### • PROCEDURE

**Buffer:** Dissolve 6.80 g of monobasic potassium phosphate in 900 mL of water, and add 1.0 mL of triethylamine. Adjust with phosphoric acid to a pH of  $6.00 \pm 0.05$ , and dilute with water to 1000 mL.

**Diluent:** Acetonitrile and water (1:1)

**Mobile phase:** Acetonitrile and Buffer (1:1)

**Standard stock solution:** 0.5 mg/mL of USP Amiodarone Hydrochloride RS in methanol

**Standard solution:** 0.1 mg/mL USP Amiodarone Hydrochloride RS in Diluent from Standard stock solution

**Sample stock solution:** 0.5 mg/mL of Amiodarone Hydrochloride in methanol

**Sample solution:** 0.1 mg/mL of Amiodarone Hydrochloride in Diluent from Sample stock solution

##### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 3.9-mm  $\times$  15-cm; 5- $\mu$ m packing L26

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

##### System suitability

**Sample:** Standard solution

##### Suitability requirements

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 1.0%

##### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of  $C_{25}H_{29}I_2NO_3 \cdot HCl$  in the portion of Amiodarone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of amiodarone in the *Sample solution*  
 $r_S$  = peak response of amiodarone in the *Standard solution*  
 $C_S$  = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of Amiodarone Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0%, on the dried basis

#### IMPURITIES

##### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.1% on a 1-g sample

#### Delete the following:

##### • HEAVY METALS

**Buffer:** Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 70% hydrochloric acid. Adjust, if necessary, with diluted hydrochloric acid or diluted ammonia solution to a pH of 3.5. Dilute with water to 100.0 mL.

**Lead standard stock solution (1000 ppm Pb):** 1.6 mg/mL of lead nitrate in water

**Lead standard solution:** 10 ppm of lead in water from Lead standard stock solution. [NOTE—Prepare immediately before use.]

**Phenolphthalein solution:** Dissolve 0.1 g of phenolphthalein in 80 mL of alcohol, and dilute with water to 100 mL.

**Thioacetamide solution:** Prepare a solution of 40 g/L of thioacetamide in water. To 0.2 mL of the freshly prepared solution, add 1 mL of a mixture of 85% glycerol, 1 M sodium hydroxide, and water (4:3:1). Heat in a water bath for 20 s.

**Sample solution:** Place about 1 g of Amiodarone Hydrochloride in a silica crucible along with 4 mL of magnesium sulfate solution (250 g/L of diluted sulfuric acid). Mix using a fine glass rod, and heat cautiously. If the mixture is a liquid, evaporate gently to dryness on a water bath. Progressively heat to ignition, and continue heating until an almost white or a mostly grayish residue is obtained. Carry out the ignition at a temperature not exceeding 800°. Allow to cool. Moisten the residue with a few drops of dilute sulfuric acid. Evaporate, ignite again, and allow to cool. The total period of ignition must not exceed 2 h. Dissolve the residue in two portions, 5 mL each, of 20% hydrochloric acid. Add 0.1 mL of Phenolphthalein solution followed by 25% ammonia water until a pink color is obtained. Cool, add glacial acetic acid until the solution is decolorized, and add 0.5 mL in excess. Filter if necessary, wash the filter, and dilute with water to 20 mL.



**Standard solution:** Proceed as directed for *Sample solution*, using 2 mL of *Lead standard solution* instead of Amiodarone Hydrochloride. To 10 mL of the solution obtained, add 2 mL of the *Sample solution*.

**Monitor solution:** Proceed as directed for *Sample solution*, adding 2 mL of *Standard solution* to 1 g of Amiodarone Hydrochloride.

**Blank solution:** 10 mL of water and 2 mL of *Sample solution*.

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, *Blank solution*, and *Monitor solution*.

To 12 mL each of the *Standard solution*, *Sample solution*, *Blank solution*, and *Monitor solution* add 2 mL of *Buffer solution*, and mix. Add 1.2 mL of *Thioacetamide solution*, and immediately mix again. Examine the solutions after 2 min. The test is invalid if the *Standard solution* does not show a slight brown color compared to the *Blank solution* or if the *Monitor solution* is not comparable with the *Standard solution*.

**Acceptance criteria:** Any brown color in the *Sample solution* is not more intense than that in the *Standard solution* (20 ppm). [NOTE—If the result is difficult to judge, pass the solutions through a membrane filter having a porosity of 3 µm. Carry out the filtration slowly and uniformly, applying moderate and constant pressure. Compare the spots on the filters obtained from the different solutions.] (Official 1-Jan-2018)

#### Organic Impurities

[NOTE—The product meets the requirements for both *Procedure 1* and *Procedure 2*.]

##### • PROCEDURE 1

**Potassium iodobismuthate solution:** Dissolve 100 g of tartaric acid in 400 mL of water, and add 8.5 g of bismuth subnitrate. Shake for 1 h, add 200 mL of a 400 g/L solution of potassium iodide, and shake well. Allow to stand for 24 h, filter, and protect from light.

**Standard solution A:** 0.02 mg/mL of USP Amiodarone Related Compound H RS in methylene chloride

**Standard solution B:** *Standard solution A* and *Sample solution* (1:1).

**Sample solution:** 100 mg/mL of Amiodarone Hydrochloride in methylene chloride

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** Suitable layer of chromatographic silica gel and fluorescent indicator with maximum absorbance at 254 nm

#### Application volume

**Standard solution A:** 50 µL

**Standard solution B:** 100 µL

**Sample solution:** 50 µL

**Developing solvent system:** Methylene chloride, methanol, and anhydrous formic acid (17:2:1)

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the plate in the *Developing solvent system* until the solvent front has moved NLT two-thirds the length of the plate, and dry in a current of cold air. Spray the plate with *Potassium iodobismuthate solution* and then with 3% hydrogen peroxide solution. Examine immediately in daylight: the spot from *Standard solution B* due to amiodarone related compound H is clearly visible.

**Acceptance criteria:** Any spot with the same  $R_f$  as the spot due to amiodarone related compound H from the *Sample solution* is not more intense than the spot from *Standard solution A* (0.02%).

##### • PROCEDURE 2

**Buffer:** Add 3 mL of glacial acetic acid to 800 mL of water. Adjust with diluted ammonia solution to a pH of 4.9, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile: methanol: *Buffer* (4:3:3 v/v/v).

**Diluent:** Acetonitrile and water (1:1)

**Standard stock solution:** Dissolve equal quantities of USP Amiodarone Related Compound D RS, USP Amiodarone Related Compound E RS, and USP Amiodarone Hydrochloride RS in a known amount of methanol.

**Standard solution:** 0.01 mg/mL each of USP Amiodarone Related Compound D RS, USP Amiodarone Related Compound E RS, and USP Amiodarone Hydrochloride RS, in *Diluent* from *Standard stock solution*

**Sample solution:** 5 mg/mL of Amiodarone Hydrochloride in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**Run time:** 2 times the retention time of amiodarone

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.5 between amiodarone related compound D and amiodarone related compound E

#### Analysis

[NOTE—Disregard any peak that is less than 0.05%.]

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Amiodarone Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of each impurity in the *Sample solution*

$r_s$  = peak response of amiodarone in the *Standard solution*

$C_s$  = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of Amiodarone Hydrochloride in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amiodarone related compound A <sup>a</sup>	0.26	0.2
Amiodarone related compound D <sup>b</sup>	0.29	0.2
Amiodarone related compound E <sup>c</sup>	0.37	0.2
Amiodarone related compound B <sup>d</sup>	0.49	0.2
Amiodarone related compound C <sup>e</sup>	0.55	0.2
Amiodarone related compound G <sup>f</sup>	0.62	0.2
Amiodarone related compound F <sup>g</sup>	0.69	0.2

<sup>a</sup> (2-Butylbenzofuran-3-yl)[4-[2-(diethylamino)ethoxy]phenyl]methanone.

<sup>b</sup> (2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl)methanone.

<sup>c</sup> (2-Butylbenzofuran-3-yl)(4-hydroxyphenyl)methanone.

<sup>d</sup> (2-Butylbenzofuran-3-yl)[4-[2-(ethylamino)ethoxy]-3,5-diiodophenyl]methanone.

<sup>e</sup> (2-Butylbenzofuran-3-yl)[4-[2-(diethylamino)ethoxy]-3-iodophenyl]methanone.

<sup>f</sup> [2-[(1R)-1-Methoxybutyl]benzofuran-3-yl][4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]methanone.

<sup>g</sup> (2-Butylbenzofuran-3-yl)(4-hydroxy-3-iodophenyl)methanone.



Impurity Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amiodarone hydrochloride	1.00	—
Any other individual impurity	—	0.10

<sup>a</sup> (2-Butylbenzofuran-3-yl)[4-[2-(diethylamino)ethoxy]phenyl]methanone.<sup>b</sup> (2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl)methanone.<sup>c</sup> (2-Butylbenzofuran-3-yl)(4-hydroxyphenyl)methanone.<sup>d</sup> (2-Butylbenzofuran-3-yl)[4-[2-(ethylamino)ethoxy]-3,5-diiodophenyl]methanone.<sup>e</sup> (2-Butylbenzofuran-3-yl)[4-[2-(diethylamino)ethoxy]-3-iodophenyl]methanone.<sup>f</sup> [2-[(1*R*S)-1-Methoxybutyl]benzofuran-3-yl][4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]methanone.<sup>g</sup> (2-Butylbenzofuran-3-yl)(4-hydroxy-3-iodophenyl)methanone.**SPECIFIC TESTS****• LIMIT OF IODIDES**

**Solution A:** Add 1.50 g of Amiodarone Hydrochloride to 40 mL of water at 80°, and shake until completely dissolved. Cool, and dilute with water to 50.0 mL.

**Standard solution:** To 15.0 mL of *Solution A* add 1.0 mL of 0.1 M hydrochloric acid, 1.0 mL of an 88.2 mg/L solution of potassium iodide, and 1.0 mL of 0.05 M potassium iodate. Dilute with water to 20.0 mL. Allow to stand protected from light for 4 h.

**Sample solution:** To 15.0 mL of *Solution A* add 1.0 mL of 0.1 M hydrochloric acid and 1.0 mL of 0.05 M potassium iodate. Dilute with water to 20.0 mL. Allow to stand protected from light for 4 h.

**Analysis:** Measure the absorbances of the *Standard solution* and the *Sample solution* at 420 nm, using a mixture of 15.0 mL of *Solution A* and 1.0 mL of 0.1 M hydrochloric acid diluted with water to 20.0 mL to serve as the blank. The absorbance of the *Sample solution* is NMT half the absorbance of the *Standard solution*.

**Acceptance criteria:** NMT 150 ppm

• **PH (791):** 3.2–3.8. Dissolve 1 g of Amiodarone Hydrochloride in water by heating at 80°. Cool, and dilute with water to 20 mL.

• **Loss on Drying (731):** Use 1 g of sample, and dry under vacuum (NMT 0.3 kPa) at 50° for 4 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in light-resistant, tight containers. Store at controlled room temperature.

**• USP REFERENCE STANDARDS (11)**

USP Amiodarone Hydrochloride RS

USP Amiodarone Related Compound D RS

(2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl)methanone.

C<sub>19</sub>H<sub>16</sub>I<sub>2</sub>O<sub>3</sub> 546.14

USP Amiodarone Related Compound E RS

(2-Butylbenzofuran-3-yl)(4-hydroxyphenyl)methanone.

C<sub>19</sub>H<sub>18</sub>O<sub>3</sub> 294.34

USP Amiodarone Related Compound H RS

2-Chloro-*N,N*-diethylethanamine.

C<sub>6</sub>H<sub>14</sub>ClN 135.64

**Amiodarone Hydrochloride Injection****DEFINITION**

Amiodarone Hydrochloride Injection is a sterile solution of Amiodarone Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of amiodarone hydrochloride (C<sub>25</sub>H<sub>29</sub>I<sub>2</sub>NO<sub>3</sub> · HCl). It may contain suitable preservatives.

**IDENTIFICATION**

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****• PROCEDURE**

**Buffer:** 1.36 g/L of monobasic potassium phosphate in water prepared as follows. To 1.36 g of monobasic potassium phosphate in a 1-L volumetric flask add about 900 mL of water and 1 mL of triethylamine. Adjust with phosphoric acid to a pH of 6.0, and dilute to volume.

**Mobile phase:** Acetonitrile and *Buffer* (800:200)

**Diluent:** Acetonitrile and water (60:40)

**Standard solution:** 0.025 mg/mL of USP Amiodarone Hydrochloride RS in *Diluent*

**Sample solution:** Nominally 0.012 mg/mL of amiodarone hydrochloride in *Diluent* from a suitable volume of Injection

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 10-cm; 5-μm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20 μL

**Run time:** NLT 2 times the retention time of amiodarone

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amiodarone hydrochloride (C<sub>25</sub>H<sub>29</sub>I<sub>2</sub>NO<sub>3</sub> · HCl) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak area of amiodarone from the *Sample solution*

*r<sub>S</sub>* = peak area of amiodarone from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of amiodarone hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**IMPURITIES****• ORGANIC IMPURITIES**

**Buffer:** 3 mL/L of glacial acetic acid in water prepared as follows. To a suitable amount of glacial acetic acid add water to fill 80% of the total volume, adjust with ammonia to a pH of 4.9, and dilute with water to volume.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (400:300:300)

**Diluent:** Acetonitrile, methanol, and water (50:30:20)

**Standard solution A:** 0.001 mg/mL of USP Amiodarone Hydrochloride RS in *Diluent*

**Standard solution B:** 0.03 mg/mL of USP Amiodarone Related Compound D RS and 2 μg/mL of USP Amiodarone Related Compound E RS in *Diluent*

**Sample solution:** Nominally 1 mg/mL of amiodarone hydrochloride in *Diluent* from a suitable volume of Injection

**Chromatographic system**

(See Chromatography (621), System Suitability.)



**Mode:** LC  
**Detector:** UV 240 nm  
**Column:** 4.6-mm × 15-cm; 5-μm packing L1  
**Column temperature:** 30°  
**Flow rate:** 1 mL/min  
**Injection volume:** 10 μL  
**Run time:** NLT 1.5 times the retention time of amiodarone for the *Standard solution*, and NLT 2 times the retention time of amiodarone for the *Sample solution*

**System suitability**

**Sample:** *Standard solution A*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 5.0%

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of amiodarone related compound D or amiodarone related compound E in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of amiodarone related compound D or amiodarone related compound E from the *Sample solution*

$r_S$  = peak area of amiodarone related compound D or amiodarone related compound E from *Standard solution B*

$C_S$  = concentration of USP Amiodarone Related Compound D RS or USP Amiodarone Related Compound E RS in *Standard solution B* (mg/mL)

$C_U$  = nominal concentration of amiodarone hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of any unspecified degradation product from the *Sample solution*

$r_S$  = peak area of amiodarone from *Standard solution A*

$C_S$  = concentration of USP Amiodarone Hydrochloride RS in *Standard solution A* (mg/mL)

$C_U$  = nominal concentration of amiodarone hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 1.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amiodarone related compound E <sup>a</sup>	0.39	0.2
Amiodarone related compound D <sup>b</sup>	0.55	3.0
Amiodarone	1.00	—
Any unspecified degradation product	—	0.20
Total impurities	—	3.5

<sup>a</sup> (2-Butylbenzofuran-3-yl)(4-hydroxyphenyl) methanone.

<sup>b</sup> (2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl) methanone.

**• LIMIT OF IODIDE**

Use freshly prepared solutions in amber glassware.

**Potassium iodate solution:** 10.7 g/L of potassium iodate in water

**Potassium iodide solution:** 88.2 mg/L of potassium iodide in water

**Amiodarone stock solution:** 5 mg/mL of amiodarone hydrochloride in water from Injection prepared by diluting 5.0 mL of Injection in a 50-mL volumetric flask to volume

**Standard solution:** 4.41 μg/mL prepared as follows.

Into a suitable flask pipet 15.0 mL of *Amiodarone stock solution*, 1.0 mL of 0.1 M hydrochloric acid, 1.0 mL of *Potassium iodide solution*, 1.0 mL of *Potassium iodate solution*, and 2.0 mL of water. Mix, and allow to stand for 4 h, protected from light.

**Sample solution:** Into a suitable flask pipet 15.0 mL of *Amiodarone stock solution*, 1.0 mL of 0.1 M hydrochloric acid, 1.0 mL of *Potassium iodate solution*, and 3.0 mL of water. Mix, and allow to stand for 4 h, protected from light.

**Blank:** Into a suitable flask pipet 15.0 mL of *Amiodarone stock solution*, 1.0 mL of 0.1 M hydrochloric acid, and 4.0 mL of water. Mix, and allow to stand for 4 h, protected from light.

**Instrumental conditions**

**Mode:** UV-Vis

**Analytical wavelength:** 420 nm

**Cell:** 1 cm

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
 Calculate the amount of iodide, in ppm, in the portion of Injection taken:

$$\text{Result} = (A_U - A_B)/[(A_S - A_B) - (A_U - A_B)] \times (C_S/C_U) \times (M_{r1}/M_{r2})$$

$A_U$  = absorbance of the *Sample solution*

$A_B$  = absorbance of the *Blank*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of potassium iodide in the *Standard solution* (μg/mL)

$C_U$  = concentration of amiodarone hydrochloride in the *Sample solution* (g/mL)

$M_{r1}$  = molecular weight of iodide, 126.90

$M_{r2}$  = molecular weight of potassium iodide, 166.00

**Acceptance criteria:** NMT 250 ppm

**OTHER COMPONENTS****• CONTENT OF BENZYL ALCOHOL** (if present)

**Internal standard solution:** 1 mg/mL of phenol in isopropyl alcohol

**Blank solution:** 0.2 mg/mL of phenol in isopropyl alcohol prepared as follows. Transfer 5 mL of *Internal standard solution* into a 25-mL volumetric flask, and dilute with isopropyl alcohol to volume.

**Standard stock solution:** 1.62 mg/mL of USP Benzyl Alcohol RS in isopropyl alcohol

**Standard solution:** Transfer 5 mL of *Internal standard solution* and 3 mL of *Standard stock solution* into a 25-mL volumetric flask, and dilute with isopropyl alcohol to volume.

**Sample stock solution:** Nominally equivalent to 1.61 mg/mL of benzyl alcohol in isopropyl alcohol prepared as follows. Transfer 2 mL of Injection into a 25-mL volumetric flask, and dilute with isopropyl alcohol to volume.

**Sample solution:** Nominally 0.2 mg/mL of phenol and 0.19 mg/mL of benzyl alcohol in isopropyl alcohol prepared as follows. Transfer 5 mL of *Internal standard solution* and 3 mL of *Sample stock solution* into a 25-mL volumetric flask, and dilute with isopropyl alcohol to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.32-mm × 30-m; coated with 1-μm phase G16



**Temperatures**

Injection port: 200°

Detector: 200°

Column: 150°

Carrier gas: Nitrogen

Flow rate: 10 mL/min

Injection volume: 1 µL

Injection type: Split injection with a split ratio, 10:1

**System suitability**Sample: *Standard solution***Suitability requirements**

Relative standard deviation: NMT 2.0% for the peak response ratio of benzyl alcohol to phenol

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzyl alcohol in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 $R_U$  = peak response ratio of benzyl alcohol to phenol from the *Sample solution* $R_S$  = peak response ratio of benzyl alcohol to phenol from the *Standard solution* $C_S$  = concentration of USP Benzyl Alcohol RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of benzyl alcohol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 8.33 USP Endotoxin Units/mg of amiodarone hydrochloride
- **STERILITY TESTS** (71): Meets the requirements
- **PH** (791): 3.0–5.0
- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS**: Meets the requirements in *Injections and Implanted Drug Products* (1)

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in single-dose or multiple-dose glass containers, protected from light and excessive heat. Store at controlled room temperature.
- **LABELING**: Label it to indicate that it is to be diluted to the appropriate strength with a suitable parenteral vehicle prior to administration. Label it to indicate the type and amount of preservative used. Label it to indicate that it is preservative free, if no preservative is present.
- **USP REFERENCE STANDARDS** (11)
  - USP Amiodarone Hydrochloride RS
  - USP Amiodarone Related Compound D RS  
(2-Butylbenzofuran-3-yl)(4-hydroxy-3,5-diiodophenyl) methanone.  
 $C_{19}H_{16}I_2O_3$  546.14
  - USP Amiodarone Related Compound E RS  
(2-Butylbenzofuran-3-yl)(4-hydroxyphenyl) methanone.  
 $C_{19}H_{18}O_3$  294.34
  - USP Benzyl Alcohol RS
  - USP Endotoxin RS

## Amiodarone Hydrochloride Compounded Oral Suspension

**DEFINITION**

Amiodarone Hydrochloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of amiodarone hydrochloride ( $C_{25}H_{29}I_2NO_3 \cdot HCl$ ). Prepare Amiodarone Hydrochloride Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Amiodarone Hydrochloride tablets*	600 mg of amiodarone hydrochloride
Vehicle: a 1:1 mixture of Ora-Sweet <sup>®</sup> (regular or sugar-free) and Ora-Plus, <sup>®</sup> a sufficient quantity to make	120 mL

\* Cordarone 200-mg tablets, Wyeth-Ayerst Laboratories, Philadelphia, PA.

<sup>®</sup> Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Amiodarone Hydrochloride tablets* in a suitable mortar and comminute to a fine powder with a pestle. Adjust the pH of the *Vehicle* to  $6.5 \pm 0.5$  with a sodium bicarbonate 50-mg/mL solution prepared in Purified Water. Add the *Vehicle* in small portions and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make an amiodarone hydrochloride liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume and mix well.

**ASSAY****• PROCEDURE**

Mobile phase: Methanol, water, and 50 mM monobasic ammonium phosphate (0.5:0.5:99)

Standard solution: 2.5 mg/mL of USP Amiodarone Hydrochloride RS in *Mobile phase*Sample solution: Shake thoroughly by hand each bottle of the Oral Suspension. Prepare 2.5 mg/mL of amiodarone hydrochloride from Oral Suspension and *Mobile phase*.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

**System suitability**Sample: *Standard solution*

[NOTE—The retention time for amiodarone is about 3.6 min.]

**Suitability requirements**

Relative standard deviation: NMT 2.1% for replicate injections

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of amiodarone hydrochloride ( $C_{25}H_{29}I_2NO_3 \cdot HCl$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Amiodarone Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of amiodarone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**SPECIFIC TESTS**

- **PH** (791): 5.8–6.8

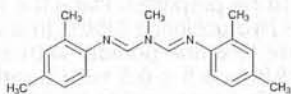
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE**: NMT 90 days after the date on which it was compounded when stored in a refrigerator; NMT 30 days when stored at controlled room temperature



- **LABELING:** Label it to state that it is to be well shaken before use and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS (11)**  
USP Amiodarone Hydrochloride RS

## Amitraz



$C_{19}H_{23}N_3$  293.41  
Methanimidamide, *N'*-(2,4-dimethylphenyl)-*N*-[[2,4-dimethylphenyl]imino]methyl]-*N*-methyl-;  
*N*-Methyl-*N'*-2,4-xylyl-*N*-(*N*-2,4-xylylformimidoyl)formamidine;  
*N*-Methylbis(2,4-xylyliminomethyl)amine [33089-61-1].

### DEFINITION

Amitraz contains NLT 95.0% and NMT 101.5% of amitraz ( $C_{19}H_{23}N_3$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197):** [NOTE—Methods described under *Infrared Absorption* (197K), (197M), or (197A) may be used.]
- **B.** The retention time of the amitraz peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Internal standard solution:** 0.7% v/v solution of squalane in methyl acetate

**Standard solution:** 5.0 mg/mL of USP Amitraz RS in *Internal standard solution*

**Sample solution:** 5.0 mg/mL of Amitraz in *Internal standard solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm × 15-m fused silica; coated with a 1.5-μm layer of liquid phase G9

#### Temperatures

**Detector:** 300°

**Inlet:** 230°

**Column:** 220°

**Carrier gas:** Helium

**Flow rate:** 12 mL/min

**Injection volume:** 1 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The elution order is amitraz followed by squalane.]

#### Suitability requirements

**Resolution:** NLT 3.0 between amitraz and squalane

**Relative standard deviation:** NMT 2.0% from the peak area ratio of amitraz to squalane

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amitraz ( $C_{19}H_{23}N_3$ ) in the portion of Amitraz taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of amitraz and squalane from the *Sample solution*

$R_S$  = peak response ratio of amitraz and squalane from the *Standard solution*

$C_S$  = concentration of USP Amitraz RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amitraz in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–101.5% on the anhydrous basis

### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.2%

#### ORGANIC IMPURITIES

**Standard solution:** 0.05 mg/mL of 2,4-dimethylaniline, 1.0 mg/mL of USP Amitraz Related Compound A RS, 0.5 mg/mL of USP Amitraz Related Compound B RS, and 1.0 mg/mL of USP Amitraz Related Compound C RS in methyl acetate

**Sample solution:** 50.0 mg/mL of Amitraz in methyl acetate

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm × 10-m fused silica; coated with a 5-μm layer of liquid phase G27

#### Temperatures

**Detector:** 300°

**Inlet:** 230°

**Column:** See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
125	0	125	5
125	5	270	15

**Carrier gas:** Helium

**Flow rate:** 12 mL/min

**Injection volume:** 1 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between amitraz related compound A and amitraz related compound B

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each of amitraz related compounds A, B, and C in the portion of Amitraz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of the corresponding related compound from the *Standard solution*

$C_S$  = concentration of the corresponding related compound in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amitraz in the *Sample solution* (mg/mL)

Calculate the percentage of 2,4-dimethylaniline and any other individual impurity in the portion of Amitraz taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of 2,4-dimethylaniline from the *Standard solution*

$C_S$  = concentration of 2,4-dimethylaniline in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amitraz in the *Sample solution* (mg/mL)



**Acceptance criteria:** See Table 2. The reporting level for impurities is 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,4-Dimethylaniline	0.11	0.1
Amitraz related compound A	0.35	2
Amitraz related compound B	0.40	1
Amitraz related compound C	0.86	2
Amitraz	1.0	—
Any other individual impurity	—	0.1

### SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 0.1%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS (11)**
  - USP Amitraz RS
  - USP Amitraz Related Compound A RS
  - 2,4-Dimethylphenyl formamide;  
N-(2,4-Dimethylphenyl)formamide.  
 $C_9H_{11}NO$  149.19
  - USP Amitraz Related Compound B RS
  - 2,4-Dimethylphenyl N-methyl-formamidine;  
N'-(2,4-Dimethylphenyl)-N-methylformimidamide.  
 $C_{10}H_{14}N_2$  162.23
  - USP Amitraz Related Compound C RS
  - Bisformamidine analog;  
N,N'-Bis(2,4-dimethylphenyl)formimidamide.  
 $C_{17}H_{20}N_2$  252.35

## Amitraz Concentrate for Dip

### DEFINITION

Amitraz Concentrate for Dip contains amitraz in a suitable vehicle. It may contain a suitable stabilizing agent. It contains NLT 90.0% and NMT 120.0% of the labeled amount of amitraz ( $C_{19}H_{23}N_3$ ).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHY

**Standard solution:** 5 mg/mL of USP Amitraz RS in toluene  
**Sample solution:** Nominally 5 mg/mL of amitraz from Concentrate for Dip diluted with toluene  
**Chromatographic system**  
 (See Chromatography (621), Thin-Layer Chromatography.)  
**Mode:** TLC  
**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture  
**Application volume:** 2  $\mu$ L  
**Developing solvent system:** Cyclohexane, ethyl acetate, and triethylamine (5:3:2)  
**Spray reagent:** 0.5% solution of N-(1-naphthyl)ethylenediamine dihydrochloride in methanol

#### Analysis

**Samples:** Standard solution and Sample solution  
 Stand the plate to a depth of 3.5 cm in a solution prepared by dissolving 35 g of acetamide in 100 mL of methanol, adding 100 mL of triethylamine, and diluting to 250 mL with methanol. Allow the wet plate to

stand in a current of cold air for 30 s. Immediately apply the Samples separately to the plate, at a level about 1 cm below the top of the impregnated zone. Promptly develop the plate until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber and allow to air-dry. Examine the plate under short-wave-length UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the Sample solution corresponds to that of the Standard solution.

- **B.** The retention time of the amitraz peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Internal standard solution:** 0.7% v/v solution of squalane in methyl acetate

**Standard solution:** 5.0 mg/mL of USP Amitraz RS in Internal standard solution

**Sample solution:** Nominally equivalent to 5.0 mg/mL of amitraz from Concentrate for Dip in Internal standard solution

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm  $\times$  15-m fused silica; coated with a 1.5- $\mu$ m layer of liquid phase G9

#### Temperatures

**Column:** 220°

**Inlet:** 230°

**Detector:** 300°

**Carrier gas:** Helium

**Flow rate:** 12 mL/min

**Injection volume:** 1  $\mu$ L

#### System suitability

**Sample:** Standard solution

[NOTE—The elution order is amitraz, followed by squalane.]

#### Suitability requirements

**Resolution:** NLT 3.0 between amitraz and squalane

**Relative standard deviation:** NMT 2.0% from the peak area ratio of amitraz to squalane

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of amitraz ( $C_{19}H_{23}N_3$ ) in the portion of Concentrate for Dip taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of amitraz and squalane from the Sample solution

$R_S$  = peak response ratio of amitraz and squalane from the Standard solution

$C_S$  = concentration of USP Amitraz RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of amitraz in the Sample solution (mg/mL)

**Acceptance criteria:** 90.0%–120.0%

### SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 0.15%

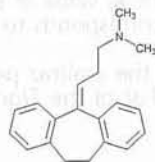
### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate that it is for veterinary use only and that it is to be diluted before use. The label also states the name and quantity of diluent to be used, the directions for dilution, and the conditions for storage of the constituted Concentrate for Dip.



- **USP REFERENCE STANDARDS** (11)  
USP Amitraz RS

## Amitriptyline Hydrochloride



$C_{20}H_{23}N \cdot HCl$  313.86  
1-Propanamine, 3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N,N-dimethyl-, hydrochloride;  
10,11-Dihydro-N,N-dimethyl-5H-dibenzo[a,d]cycloheptene- $\Delta^{5,7}$ -propylamine hydrochloride [549-18-8].

### DEFINITION

Amitriptyline Hydrochloride contains NLT 98.0% and NMT 102.0% of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (17K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

### ASSAY

#### • PROCEDURE

**Diluted phosphoric acid:** Phosphoric acid and water (1:10)

**Buffer:** 1.42 g/L of dibasic sodium phosphate ( $Na_2HPO_4$ ) in water, adjusted with *Diluted phosphoric acid* to a pH of 7.7

**Mobile phase:** Methanol and *Buffer* (7:3)

**System suitability stock solution A:** 1 mg/mL of USP Amitriptyline Related Compound A RS in methanol

**System suitability stock solution B:** 0.4 mg/mL of USP Amitriptyline Hydrochloride RS, 0.6 mg/mL each of USP Amitriptyline Related Compound B RS, USP Cyclobenzaprine Hydrochloride RS, and USP Nortriptyline Hydrochloride RS in *Mobile phase*

**Standard solution:** 0.2 mg/mL of USP Amitriptyline Hydrochloride RS in *Mobile phase*

**System suitability solution:** 1  $\mu$ g/mL of amitriptyline hydrochloride, 0.5  $\mu$ g/mL of amitriptyline related compound A, and 1.5  $\mu$ g/mL each of amitriptyline related compound B, cyclobenzaprine hydrochloride, and nortriptyline hydrochloride from suitable volumes of *Standard solution*, *System suitability stock solution A*, and *System suitability stock solution B* in *Mobile phase*

**Sample solution:** 0.2 mg/mL of Amitriptyline Hydrochloride in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Column temperature:** 45°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**Run time:** 1.5 times the retention time of amitriptyline

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—For relative retention times, see *Table 1*.]

#### Suitability requirements

**Resolution:** NLT 1.5 between amitriptyline related compound B and nortriptyline, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the amitriptyline peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ) in the portion of Amitriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amitriptyline Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

#### Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1-Jan-2018)

#### • ORGANIC IMPURITIES

Diluted phosphoric acid, Buffer, Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

**Standard solution:** Use the *System suitability solution*, prepared as directed in the *Assay*.

**Sample solution:** 1 mg/mL of Amitriptyline Hydrochloride in *Mobile phase*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentages of the individual amitriptyline related compounds in the portion of Amitriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each amitriptyline related compound from the *Sample solution*

$r_S$  = peak response of the corresponding amitriptyline related compound from the *Standard solution*

$C_S$  = concentration of the corresponding amitriptyline related compound in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amitriptyline Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Amitriptyline Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity from the *Sample solution*

$r_S$  = peak response of USP Amitriptyline Hydrochloride RS from the *Standard solution*

$C_S$  = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amitriptyline Hydrochloride in the *Sample solution* (mg/mL)

[NOTE—Discard any peak with a relative retention time less than 0.22.]



Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amitriptyline related compound A	0.35	0.05
Amitriptyline related compound B	0.52	0.15
Nortriptyline	0.60	0.15
Cyclobenzaprine	0.76	0.15
Amitriptyline	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

### SPECIFIC TESTS

#### • PH (791)

Sample: 10 mg/mL in water

Acceptance criteria: 5.0–6.0, in a solution (1 in 100)

#### • LOSS ON DRYING (731)

Analysis: Dry a sample at a pressure not exceeding 5 mm of mercury at 60° to constant weight.

Acceptance criteria: NMT 0.5%

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in well-closed containers.

#### • USP REFERENCE STANDARDS (11)

USP Amitriptyline Hydrochloride RS

USP Amitriptyline Related Compound A RS

10,11-Dihydro-5H-dibenzo[a,d]cyclohepten-5-one; (also known as dibenzosuberone).

C<sub>15</sub>H<sub>12</sub>O 208.26

USP Amitriptyline Related Compound B RS

5-[3-(Dimethylamino)propyl]-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ol; (also known as amitriptynol).

C<sub>20</sub>H<sub>25</sub>NO 295.42

USP Cyclobenzaprine Hydrochloride RS

USP Nortriptyline Hydrochloride RS

## Amitriptyline Hydrochloride Injection

### DEFINITION

Amitriptyline Hydrochloride Injection is a sterile solution of Amitriptyline Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of amitriptyline hydrochloride (C<sub>20</sub>H<sub>23</sub>N · HCl).

### IDENTIFICATION

#### • A.

Sample solution: Pipet 1 mL of Injection into a 125-mL separator containing 10 mL of water and 1 mL of 1 N sodium hydroxide, mix, extract with two 10-mL portions of methylene chloride, and evaporate the extracts on a steam bath just to dryness. Dissolve the residue in methanol, add 1 mL of 1.2 N hydrochloric acid, and then add methanol to make 100 mL. Dilute 10 mL of this solution with methanol to 100 mL.

Acceptance criteria: The UV absorption spectrum of this solution exhibits a maximum at the same wavelength as that of a similar solution of USP Amitriptyline Hydrochloride RS, concomitantly measured.

#### • B. The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

Buffer: Dissolve 11.04 g of monobasic sodium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 2.5 ± 0.5, and dilute with water to 1000 mL.

Mobile phase: Acetonitrile and Buffer (42:58)

Standard solution: 0.2 mg/mL of USP Amitriptyline Hydrochloride RS in water

Sample solution: Nominally 0.2 mg/mL of amitriptyline hydrochloride from a suitable volume of the Injection in water

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

#### System suitability

Sample: Standard solution

Suitability requirements

Column efficiency: NLT 800 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of amitriptyline hydrochloride (C<sub>20</sub>H<sub>23</sub>N · HCl) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Amitriptyline Hydrochloride RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of amitriptyline hydrochloride in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

### SPECIFIC TESTS

#### • PYROGEN TEST (151)

Sample: Amitriptyline Hydrochloride Injection, diluted with Sodium Chloride Injection containing 0.9% of sodium chloride to a concentration of 2.5 mg of amitriptyline hydrochloride/mL

Acceptance criteria: Meets the requirements for a test dose of 1 mL/kg

#### • PH (791): 4.0–6.0

#### • OTHER REQUIREMENTS: Meets the requirements in Injections and Implanted Drug Products (1)

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

#### • USP REFERENCE STANDARDS (11)

USP Amitriptyline Hydrochloride RS

## Amitriptyline Hydrochloride Tablets

### DEFINITION

Amitriptyline Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of amitriptyline hydrochloride (C<sub>20</sub>H<sub>23</sub>N · HCl).

### IDENTIFICATION

#### • A.

Sample stock solution: Nominally 0.1 mg/mL of amitriptyline hydrochloride in methanol from a suitable



amount of finely powdered Tablets. Filter a portion of the solution, and use the filtrate.

**Sample solution:** Nominally 0.01 mg/mL of amitriptyline hydrochloride from *Sample stock solution* in methanol

**Acceptance criteria:** The UV absorption spectrum of this solution exhibits a maximum at the same wavelength as that of a similar solution of USP Amitriptyline Hydrochloride RS, concomitantly measured.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

## ASSAY

### • PROCEDURE

**Buffer:** 11.04 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of  $2.5 \pm 0.5$ , and dilute to make 1000 mL.

**Mobile phase:** Acetonitrile and *Buffer* (42:58)

**Standard solution:** 0.2 mg/mL of USP Amitriptyline Hydrochloride RS in *Mobile phase*

**Sample solution:** Nominally 0.2 mg/mL of amitriptyline hydrochloride in *Mobile phase* prepared as follows.

Transfer NLT 20 Tablets to a suitable volumetric flask, add 50% of the flask volume of *Mobile phase*, and shake the mixture for 1 h or until the Tablets have disintegrated. Dilute with *Mobile phase* to volume, and filter. Dilute the clear filtrate with *Mobile phase* to obtain a solution with a nominal concentration of 0.2 mg/mL of amitriptyline hydrochloride.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Column efficiency:** NLT 800 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ) in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amitriptyline hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

### Instrumental conditions

**Analytical wavelength:** UV 239 nm

**Standard solution:** USP Amitriptyline Hydrochloride RS in *Medium*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ) dissolved from UV absorbances of the *Sample solution*, suitably

diluted with *Medium* if necessary, in comparison with a *Standard solution* having a known concentration.

**Tolerances:** NLT 75% (Q) of the labeled amount of amitriptyline hydrochloride ( $C_{20}H_{23}N \cdot HCl$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**  
USP Amitriptyline Hydrochloride RS

## Amlodipine Compounded Oral Suspension

### DEFINITION

Amlodipine Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ).

Prepare Amlodipine Compounded Oral Suspension 1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Amlodipine besylate tablets <sup>a</sup> equivalent to	100 mg of amlodipine
Vehicle: a 1:1 mixture of Ora-Sweet <sup>b</sup> and Ora-Plus, <sup>b</sup> a sufficient quantity to make	100 mL

<sup>a</sup> Norvasc 5-mg tablets, Pfizer, Inc., Groton, CT.

<sup>b</sup> Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of tablets in a suitable mortar and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make an amlodipine liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well. [NOTE—To ensure component uniformity, homogenization is recommended.]

## ASSAY

### • PROCEDURE

**Mobile phase:** Acetonitrile, methanol, and 40 mM ammonium acetate (50:15:35). Filter through a nylon 66 filter of 0.45- $\mu$ m pore size, and degas.

**Standard stock solution:** Dissolve an appropriately weighed amount of USP Amlodipine Besylate RS in methanol, equivalent to 1.0 mg/mL of amlodipine (approximately equal to 1.4 mg/mL of amlodipine besylate).

**Standard solution:** Transfer 1.0 mL of the *Standard stock solution* into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of about 20  $\mu$ g/mL of amlodipine. Centrifuge.

**Sample solution:** Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of Oral Suspension into a 50-mL volumetric flask, rinse the pipet three times with *Mobile phase*, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of about 20  $\mu$ g/mL of amlodipine. Centrifuge.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 240 nm  
 Column: 3.0-mm × 15-cm; 5-μm packing L10  
 Flow rate: 0.4 mL/min  
 Injection volume: 10 μL

#### System suitability

Sample: *Standard solution*

[NOTE—The retention time for amlodipine is about 10.1 min.]

#### Suitability requirements

Column efficiency: NLT 4000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of amlodipine from the *Sample solution*

$r_S$  = peak response of amlodipine from the *Standard solution*

$C_S$  = concentration of amlodipine in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of amlodipine in the *Sample solution* (μg/mL)

Acceptance criteria: 90.0%–110.0%

#### SPECIFIC TESTS

- **PH (791):** 4.0–5.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator; NMT 60 days when stored at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS (11)**  
 USP Amlodipine Besylate RS

## Amlodipine and Benazepril Hydrochloride Capsules

#### DEFINITION

Amlodipine and Benazepril Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of each of amlodipine ( $C_{20}H_{25}N_2O_5Cl$ ) and benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ).

#### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

**Buffer 1:** 0.7% (v/v) of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0, and add 1.2 g of tetrabutyl ammonium hydrogen sulfate per 1 L of *Buffer*. Pass through a suitable filter of 0.45-μm pore size.

**Buffer 2:** 7.0 mL/L of triethylamine in water. Adjust with phosphoric acid to a pH of 3.0. Pass through a suitable filter of 0.45-μm pore size.

**Diluent:** Acetonitrile, methanol, and *Buffer 2* (20:30:50)

**Mobile phase:** Acetonitrile, methanol, and *Buffer 1* (10:30:70)

**Standard solution:** Prepare the corresponding solutions in *Diluent* as directed in Table 1.

Table 1

Strength of Capsule (Amlodipine (mg)/ Benazepril Hydrochloride (mg))	Concentration of Amlodipine Besylate/Benazepril Hydrochloride (mg/mL)
2.5/10	0.18/0.5
5/20	0.18/0.5
5/10	0.18/0.25
10/20	0.36/0.5
5/40 and 10/40	0.04/0.16

Pass through a suitable membrane filter of 0.45-μm pore size.

**Sample solution:** Transfer the contents of five Capsules into a volumetric flask as given in Table 2. Add *Diluent* (about 70% of the volume of the flask) and keep on a rotary shaker for about 45 min, sonicate for 30 min with occasional shaking, and dilute with *Diluent* to volume. Centrifuge a portion of the above solution at 3000 rpm for 10 min, and pass through a filter of 0.45-μm pore size.

Table 2

Strength of Capsule (Amlodipine (mg)/ Benazepril Hydrochloride (mg))	Concentration of Amlodipine/Benazepril Hydrochloride (mg/mL)
2.5/10	0.125/0.5
5/20	0.125/0.5
5/10	0.125/0.25
10/20	0.25/0.5
5/40	0.02/0.16
10/40	0.04/0.16

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 237 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1.2 mL/min

Injection volume: 10 μL

Run time: Two times the retention time of amlodipine

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Tailing factor: NMT 2.0 for both amlodipine and benazepril peaks

Relative standard deviation: NMT 2.0% for both amlodipine and benazepril peaks

#### Analysis

Samples: *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}N_2O_5Cl$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = response of the amlodipine peak from the *Sample solution*

$r_S$  = response of the amlodipine peak from the *Standard solution*

$C_S$  = concentration of amlodipine in the *Standard solution* (mg/mL)



- $C_U$  = nominal concentration of amlodipine in the Sample solution (mg/mL)  
 $M_{r1}$  = molecular weight of amlodipine, 408.88  
 $M_{r2}$  = molecular weight of amlodipine besylate, 567.05

Calculate the percentage of the labeled amount of benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ), in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = response of the benazepril peak from the Sample solution  
 $r_S$  = response of the benazepril peak from the Standard solution  
 $C_S$  = concentration of benazepril hydrochloride in the Standard solution (mg/mL)  
 $C_U$  = nominal concentration of benazepril hydrochloride in the Sample solution (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of amlodipine free base and 90.0%–110.0% of the labeled amount of benazepril hydrochloride

## PERFORMANCE TESTS

### • DISSOLUTION (711)

**Medium:** 0.01 N hydrochloric acid; 500 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Buffer:** 2.72 g/L of potassium dihydrogen phosphate in water. Add 0.2% (v/v) of triethylamine per L. Adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Acetonitrile, methanol, and Buffer (15:35:50)

**Amlodipine besylate standard stock solution:**

0.385 mg/mL of USP Amlodipine Besylate RS in Medium

**Benazepril hydrochloride standard stock solution:**

0.225 mg/mL of USP Benazepril Hydrochloride RS in Medium

**Standard solution:** Dilute aliquots of the Amlodipine besylate standard stock solution and Benazepril hydrochloride standard stock solution with Medium as per Table 3.

Table 3

Strength of the Capsule (Amlodipine/Benazepril Hydrochloride) (mg/mg)	Volume of Amlodipine Besylate Standard Stock Solution/Volume of Benazepril Hydrochloride Standard Stock Solution (mL)	Volume of the Flask (mL)
2.5/10	1/5	50
5/10	2/5	50
5/20	2/10	50
10/20	2/5	25

For Tablet strengths 5/40 (Amlodipine/Benazepril Hydrochloride, mg/mg): 0.01 mg/mL of USP Amlodipine RS and 0.08 mg/mL of USP Benazepril Hydrochloride RS in Medium.

For Tablet strengths 10/40 (Amlodipine/Benazepril Hydrochloride, mg/mg): 0.02 mg/mL of USP Amlodipine RS and 0.08 mg/mL of USP Benazepril Hydrochloride RS in Medium.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 237 nm

**Column:** 4.6-mm  $\times$  10-cm; 3- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 50  $\mu$ L

### System suitability

**Sample:** Standard solution

### Suitability requirements

**Tailing factor:** NMT 2.0 for both amlodipine and benazepril peaks

**Relative standard deviation:** NMT 2.0% for both amlodipine and benazepril peaks

### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}N_2O_5Cl$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times 100$$

- $r_U$  = peak response for amlodipine from the Sample solution  
 $r_S$  = peak response for amlodipine from the Standard solution  
 $C_S$  = concentration of USP Amlodipine Besylate RS in the Standard solution  
 $L$  = label claim for amlodipine (mg/Capsule)  
 $M_{r1}$  = molecular weight of amlodipine, 408.88  
 $M_{r2}$  = molecular weight of amlodipine besylate, 567.05  
 $V$  = volume of Medium, 500 mL

Calculate the percentage of the labeled amount of benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

- $r_U$  = peak response for benazepril from the Sample solution  
 $r_S$  = peak response for benazepril from the Standard solution  
 $C_S$  = concentration of benazepril hydrochloride in the Standard solution  
 $L$  = label claim for benazepril hydrochloride (mg/Capsule)  
 $V$  = volume of Medium, 500 mL

**Tolerances:** NLT 80% (Q) of the labeled amounts of amlodipine ( $C_{20}H_{25}N_2O_5Cl$ ) and benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### • PROCEDURE

**Buffer 1, Buffer 2, and Diluent:** Proceed as directed in the Assay.

**Solution A:** Acetonitrile and Buffer 1 (20:80)

**Solution B:** Methanol and Buffer 1 (80:20)

**Mobile phase:** See Table 4.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	85	15
100	30	70
101	85	15
110	85	15

**Standard stock solution:** 0.36 mg/mL of amlodipine and amlodipine related compound A and 1 mg/mL each of benazepril hydrochloride and benazepril related compound C solution in Diluent

**Standard solution:** 1  $\mu$ g/mL of amlodipine and amlodipine related compound A and 3  $\mu$ g/mL each of benazepril hydrochloride and benazepril related compound C solution in Diluent



**Sample solution:** 0.25 mg/mL of amlodipine from powdered Capsules (NLT 20). [NOTE—The benazepril hydrochloride concentration may vary depending on the ratio of amlodipine to benazepril hydrochloride in the Capsule.] Initially add *Diluent*, about 70% of the volume of the flask, sonicate for 30 min with intermittent shaking, and dilute with *Diluent* to volume. Pass through a membrane filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 237 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.2 mL/min

**Injection volume:** 40  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for both amlodipine and benazepril peaks

**Resolution:** NLT 2.0 between the amlodipine and benazepril peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amlodipine related compound A in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = response of amlodipine related compound A from the *Sample solution*

$r_S$  = response of amlodipine related compound A from the *Standard solution*

$C_S$  = concentration of amlodipine related compound A in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of amlodipine related compound A, 408.88

$M_{r2}$  = molecular weight of amlodipine related compound A fumarate, 522.93

Calculate the percentage of benazepril related compound C in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = response of benazepril related compound C from the *Sample solution*

$r_S$  = response of benazepril related compound C from the *Standard solution*

$C_S$  = concentration of benazepril related compound C in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benazepril hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = response of each other impurity from the *Sample solution*

$r_T$  = sum of responses of all peaks from the *Sample solution*

**Acceptance criteria:** See *Table 5*.

**Table 5**

Impurity Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benazepril related compound C <sup>a</sup>	0.23	3.0
Amlodipine related compound A <sup>b</sup>	0.44	1.0
Amlodipine	1.00	—
Benazepril	1.20	—
Any other individual unspecified impurity	—	0.2
Total impurities <sup>c</sup>	—	5.0

[NOTE—Disregard the peaks at relative retention times of 0.09 and 0.10.]

<sup>a</sup> [3-(1-Carboxy-3-phenyl-(1*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine]-1-acetic acid.

<sup>b</sup> 3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].

<sup>c</sup> Total impurities include the sum of all impurities. The process-related impurities are not included.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Amlodipine Besylate RS

USP Amlodipine Related Compound A RS

3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.

C<sub>20</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>5</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub> 522.93

USP Benazepril Hydrochloride RS

USP Benazepril Related Compound C RS

3-(1-Carboxy-3-phenyl-1(*S*)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1*H*-1-(3*S*)-benzazepine-1-acetic acid.

C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>5</sub> 396.44

## Amlodipine and Valsartan Tablets

### DEFINITION

#### Change to read:

Amlodipine and Valsartan Tablets contain <sup>o</sup>NLT 90.0% and NMT 110.0% <sup>o</sup>(RB 1-Jun-2016) of the labeled amount of amlodipine (C<sub>20</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>5</sub>) and valsartan (C<sub>24</sub>H<sub>29</sub>N<sub>5</sub>O<sub>3</sub>).

### IDENTIFICATION

- **A.** The UV absorption spectra of the major peaks of *Sample solution A* and *Sample solution B* and those of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.
- **B.** The retention times of the major peaks of *Sample solution A* and *Sample solution B* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### Change to read:

#### PROCEDURE

**Solution A:** Water and triethylamine (1000:10). Adjust with phosphoric acid to a pH of 2.8.

**Solution B:** Methanol and acetonitrile (700:300)

**Mobile phase:** See *Table 1*.



Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50
3	50	50
15	30	70
20	30	70
20.1	50	50
25	50	50

**Diluent:** Solution A and Solution B (50:50)

**Standard solution:** 0.14 mg/mL of USP Amlodipine Besylate RS and 0.16 mg/mL of USP Valsartan RS. Add methanol to 5% of the final volume to dissolve, and dilute with Diluent to volume.

**Sample stock solution:** Transfer NLT 10 Tablets into a suitable volumetric flask. Initially add water to 10% of the final volume, and sonicate to disperse as needed. Add Diluent, using about 70% of the final volume, and shake for up to 45 min to disperse. Following dispersion, sonicate for 15 min, and shake for 30 min. Dilute with Diluent to volume to obtain a solution containing known nominal concentrations of 0.1–0.2 mg/mL of amlodipine and 1.6–6.4 mg/mL of valsartan. Centrifuge the solution for about 10 min at 3000 rpm.

**Sample solution A:** Nominally equivalent to 0.1 mg/mL of amlodipine in Diluent from Sample stock solution

**Sample solution B:** Nominally equivalent to 0.16 mg/mL of valsartan in Diluent from Sample stock solution

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector**

**Assay:** UV 237 nm

**Identification A:** Diode array, •UV 200–400 nm• (RB 1-jun-2016)

**Column:** 3.9-mm × 15-cm; 5-μm packing L1

**Temperatures**

**Autosampler:** 10°

**Column:** 30°

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** Standard solution

**Suitability requirements**

**Tailing factor:** NMT 1.5 for both amlodipine and valsartan

**Relative standard deviation:** NMT 2.0% for amlodipine and valsartan

#### Analysis

**Samples:** Standard solution, Sample solution A, and Sample solution B

Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of amlodipine from Sample solution A

$r_S$  = peak response of amlodipine from the Standard solution

$C_S$  = concentration of USP Amlodipine Besylate RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of amlodipine in Sample solution A (mg/mL)

$M_{r1}$  = molecular weight of amlodipine, 408.88

$M_{r2}$  = molecular weight of amlodipine besylate, 567.05

Calculate the percentage of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of valsartan from Sample solution B

$r_S$  = peak response of valsartan from the Standard solution

$C_S$  = concentration of USP Valsartan RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of valsartan in Sample solution B (mg/mL)

**Acceptance criteria:** •90.0%–110.0%• (RB 1-jun-2016)

## PERFORMANCE TESTS

### Change to read:

#### • DISSOLUTION <711>

**Test 1•** (RB 1-jun-2016)

**Buffer:** Dissolve 6.805 g of monobasic potassium phosphate and 0.896 g of sodium hydroxide in water, and dilute with water to 1000 mL. Adjust with 0.2 N sodium hydroxide or 1 M phosphoric acid to a pH of 6.8.

**Medium:** Buffer; 1000 mL

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Mobile phase:** Acetonitrile, water, and trifluoroacetic acid (500:500:2)

**Diluent:** 1 mg/mL of polysorbate 80 in Buffer

**System suitability solution:** 0.4 mg/mL each of USP Amlodipine Besylate RS and USP Valsartan RS prepared as follows. Initially dissolve in methanol to 40% of the total volume, and dilute with Buffer to volume.

**Standard stock solution A:** 0.072 mg/mL of USP Amlodipine Besylate RS prepared as follows. Initially dissolve in methanol to 4% of the final volume, and dilute with Diluent to volume.

**Standard stock solution B:** 2.2 mg/mL of USP Valsartan RS in methanol

**Standard solution:** ( $L_1/1000$ ) mg/mL of amlodipine and ( $L_2/1000$ ) mg/mL of valsartan in Diluent from Standard stock solution A and Standard stock solution B, where  $L_1$  is the label claim of amlodipine in mg/Tablet, and  $L_2$  is the label claim of valsartan in mg/Tablet.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size. Discard the first 10 mL of the filtrate.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 15-cm; 4-μm packing L11

**Column temperature:** 40°

**Flow rate:** 1.2 mL/min

**Injection volume:** 10 μL

**Run time:** NLT 2 times the retention time of amlodipine

#### System suitability

**Samples:** System suitability solution and Standard solution

#### Suitability requirements

**Resolution:** NLT 2.0 between amlodipine and valsartan, System suitability solution

**Tailing factor:** NMT 2.0 for amlodipine and valsartan, Standard solution

**Relative standard deviation:** NMT 2.0% for amlodipine and valsartan, Standard solution

#### Analysis

**Samples:** Standard solution and Sample solution  
Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L_1) \times 100$$

$r_U$  = peak response of amlodipine from the Sample solution



$r_s$  = peak response of amlodipine from the *Standard solution*  
 $C_s$  = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 1000 mL  
 $M_{r1}$  = molecular weight of amlodipine, 408.88  
 $M_{r2}$  = molecular weight of amlodipine besylate, 567.05

$L_1$  = label claim for amlodipine (mg/Tablet)  
 Calculate the percentage of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times (1/L_2) \times 100$$

$r_u$  = peak response of valsartan from the *Sample solution*  
 $r_s$  = peak response of valsartan from the *Standard solution*  
 $C_s$  = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 1000 mL  
 $L_2$  = label claim for valsartan (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) and valsartan ( $C_{24}H_{29}N_5O_3$ ) is dissolved.

• **Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium and Time:** Proceed as directed in *Dissolution Test 1*; 1000 mL.

**Apparatus 2:** 50 rpm

**Buffer:** Mix 7.0 mL of triethylamine with 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.

**Solution A:** Acetonitrile and *Buffer* (10:90)

**Solution B:** Acetonitrile and *Buffer* (90:10)

**Mobile phase:** See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
7	30	70
8	80	20
10	80	20

**Standard stock solution A:** 0.14 mg/mL of USP Amlodipine Besylate RS prepared as follows. Initially dissolve in 10% of the final volume of methanol, and dilute with *Medium* to volume.

**Standard stock solution B:** 1.6 mg/mL of USP Valsartan RS in methanol

**Standard solution:** ( $L_1/1000$ ) mg/mL of amlodipine and ( $L_2/1000$ ) mg/mL of valsartan in *Diluent* from *Standard stock solution A* and *Standard stock solution B*, where  $L_1$  is the label claim of amlodipine in mg/Tablet, and  $L_2$  is the label claim of valsartan in mg/Tablet.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 1- $\mu$ m pore size.

**Chromatographic system**  
 (See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 237 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Temperatures**

**Autosampler:** 10°

**Column:** 50°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0 for amlodipine and valsartan

**Relative standard deviation:** NMT 2.0% for amlodipine and valsartan

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times (M_{r1}/M_{r2}) \times (1/L_1) \times 100$$

$r_u$  = peak response of amlodipine from the *Sample solution*

$r_s$  = peak response of amlodipine from the *Standard solution*

$C_s$  = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 1000 mL

$M_{r1}$  = molecular weight of amlodipine, 408.88

$M_{r2}$  = molecular weight of amlodipine besylate, 567.05

$L_1$  = label claim for amlodipine (mg/Tablet)

Calculate the percentage of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times (1/L_2) \times 100$$

$r_u$  = peak response of valsartan from the *Sample solution*

$r_s$  = peak response of valsartan from the *Standard solution*

$C_s$  = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 1000 mL

$L_2$  = label claim for valsartan (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) and NLT 80% (Q) of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) is dissolved.

**Test 3:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 3*.

**Medium, Apparatus 2, and Time:** Proceed as directed in *Dissolution Test 1*.

**Solution A:** Acetonitrile, trifluoroacetic acid, and water (10:0.1:90)

**Solution B:** Acetonitrile, trifluoroacetic acid, and water (90:0.1:10)

**Mobile phase:** See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0.01	90	10
2.5	10	90
3.0	90	10
5.0	90	10

**Diluent:** Acetonitrile and water (50:50)

**Standard stock solution A:** 0.14 mg/mL of USP Amlodipine Besylate RS prepared as follows. Initially dissolve in *Diluent* about 4% of the final volume, and dilute with *Medium* to volume.

**Standard stock solution B:** 1.6 mg/mL of USP Valsartan RS prepared as follows. Initially dissolve in about 20% of the final volume of *Diluent*, and dilute with *Medium* to volume.

**Standard solution:** ( $L_1/1000$ ) mg/mL of amlodipine and ( $L_2/1000$ ) mg/mL of valsartan in *Medium* from *Standard stock solution A* and *Standard stock solution B*, where  $L_1$  is the label claim of amlodipine in mg/Tablet, and  $L_2$  is the label claim of valsartan in mg/Tablet.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size and discard the first few mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 237 nm for amlodipine and UV 270 nm for valsartan

Column: 4.6-mm × 10-cm; 5-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μL

#### System suitability

Sample: Standard solution

#### Suitability requirements

Tailing factor: NMT 2.0 for amlodipine and valsartan

Relative standard deviation: NMT 2.0% for amlodipine and valsartan

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L_1) \times 100$$

$r_U$  = peak response of amlodipine from the Sample solution

$r_S$  = peak response of amlodipine from the Standard solution

$C_S$  = concentration of USP Amlodipine Besylate RS in the Standard solution (mg/mL)

$V$  = volume of Medium, 1000 mL

$M_{r1}$  = molecular weight of amlodipine, 408.88

$M_{r2}$  = molecular weight of amlodipine besylate, 567.05

$L_1$  = label claim for amlodipine (mg/Tablet)

Calculate the percentage of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_2) \times 100$$

$r_U$  = peak response of valsartan from the Sample solution

$r_S$  = peak response of valsartan from the Standard solution

$C_S$  = concentration of USP Valsartan RS in the Standard solution (mg/mL)

$V$  = volume of Medium, 1000 mL

$L_2$  = label claim for valsartan (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) and NLT 80% (Q) of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) is dissolved.

• (RB 1-Jun-2016)

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### Change to read:

#### • ORGANIC IMPURITIES

Mobile phase, Diluent, Sample solution A, Sample solution B, and Chromatographic system: Proceed as directed in the Assay.

Standard stock solution A: Prepare as directed for the Standard solution in the Assay.

System suitability solution: Dissolve a suitable quantity of USP Valsartan Related Compound B RS in Standard stock solution A to obtain a solution containing 0.08 mg/mL of USP Valsartan Related Compound B RS, 0.14 mg/mL of USP Amlodipine Besylate RS, and 0.16 mg/mL of USP Valsartan RS.

Sensitivity solution: 0.14 μg/mL of USP Amlodipine Besylate RS and 0.16 μg/mL of USP Valsartan RS in Diluent from Standard stock solution A

Standard stock solution B: 0.1 mg/mL of USP Amlodipine Related Compound A RS as free base prepared as follows. Add methanol to 5% of the final volume to dissolve, and dilute with Diluent to volume.

Standard solution: 0.0005 mg/mL of USP Amlodipine Related Compound A RS as free base, and 0.0003 mg/mL each of USP Amlodipine Besylate RS and USP Valsartan RS in Diluent from Standard stock solution A and Standard stock solution B, respectively

#### System suitability

Samples: System suitability solution, Sensitivity solution, and Standard solution

#### Suitability requirements

Resolution: More than 4.0 between amlodipine and valsartan related compound B and more than 4.0 between valsartan related compound B and valsartan, System suitability solution

Relative standard deviation: NMT 5.0% for amlodipine related compound A, amlodipine, and valsartan, Standard solution

Signal-to-noise ratio: NLT 10 for amlodipine and valsartan, Sensitivity solution

#### Analysis

Samples: Sample solution A, Sample solution B, and Standard solution

Calculate the percentage of amlodipine related compound A free base in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of amlodipine related compound A from Sample solution A

$r_S$  = peak response of amlodipine related compound A from the Standard solution

$C_S$  = concentration of USP Amlodipine Related Compound A RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of amlodipine in Sample solution A (mg/mL)

$M_{r1}$  = molecular weight of amlodipine related compound A free base, 406.86

$M_{r2}$  = molecular weight of amlodipine related compound A fumarate, 522.93

Calculate the percentage of valsartan related degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of valsartan related degradation product from Sample solution B

$r_S$  = peak response of valsartan from the Standard solution

$C_S$  = concentration of USP Valsartan RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of valsartan in Sample solution B (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of each unspecified degradation product from Sample solution A

$r_S$  = peak response of amlodipine from the Standard solution

$C_S$  = concentration of USP Amlodipine Besylate RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of amlodipine in Sample solution A (mg/mL)

$M_{r1}$  = molecular weight of amlodipine, 408.88

$M_{r2}$  = molecular weight of amlodipine besylate, 567.05

Acceptance criteria: See Table 4. Disregard valsartan related compound B, the benzenesulfonic acid peak at relative retention time 0.19, and any peaks below 0.1%.



Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Devaleryl valsartan <sup>a</sup>	0.24	0.2
Amlodipine related compound A <sup>b</sup>	0.50	0.5
Valsartan related degradation product 1 <sup>c</sup>	0.54	0.2
Valsartan related degradation product 2 <sup>c</sup>	0.81	0.2
Amlodipine	1.00	—
Valsartan related compound B <sup>d</sup>	1.34	—
Valsartan related degradation product 3 <sup>c</sup>	1.44	0.2
Valsartan	1.74	—
Valsartan related degradation product 4 <sup>c</sup>	2.06	0.2
Valsartan ethyl ester <sup>e</sup>	2.32	0.2
Any other unspecified degradation product	—	0.2
Total degradation products	—	1.2% (RB 1-Jun-2016)

<sup>a</sup> N-[[2'-(1H-tetrazole-5-yl)biphenyl-4-yl]methyl]-L-valine.<sup>b</sup> 3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].<sup>c</sup> These are specified unidentified degradation products. No information is available about chemical structures or chemical names for these impurities.<sup>d</sup> N-Butyryl-N-[[2'-(1H-tetrazole-5-yl)biphenyl-4-yl]methyl]-L-valine.<sup>e</sup> N-Valeryl-N-[[2'-(1H-tetrazole-5-yl)biphenyl-4-yl]methyl]-L-valine ethyl ester.**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Store at controlled room temperature, in tight containers, and in a dry place.

**Add the following:**

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. (RB 1-Jun-2016)
- **USP REFERENCE STANDARDS (11)**
  - USP Amlodipine Besylate RS
  - USP Amlodipine Related Compound A RS
  - 3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.
  - C<sub>20</sub>H<sub>23</sub>ClN<sub>2</sub>O<sub>5</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub> 522.93
  - USP Valsartan RS
  - USP Valsartan Related Compound B RS
  - N-Butyryl-N-[[2'-(1H-tetrazole-5-yl)biphenyl-4-yl]methyl]-L-valine.
  - C<sub>23</sub>H<sub>27</sub>N<sub>5</sub>O<sub>3</sub> 421.49

## Amlodipine, Valsartan, and Hydrochlorothiazide Tablets

**DEFINITION****Change to read:**

Amlodipine, Valsartan, and Hydrochlorothiazide Tablets contain  $\bullet$ NLT 92.5% and NMT 107.5% (RB 1-Jun-2016) each of the labeled amounts of amlodipine (C<sub>20</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>5</sub>), valsartan (C<sub>24</sub>H<sub>29</sub>N<sub>5</sub>O<sub>3</sub>), and hydrochlorothiazide (C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>).

**IDENTIFICATION**

- **A.** The UV absorption spectra of the amlodipine, valsartan, and hydrochlorothiazide peaks of *Sample solution A*, *Sample solution B*, and *Sample solution C* and those of the *Standard solution* exhibit maxima and minima at the same wavelengths, as obtained in the *Assay*.
- **B.** The retention times of the amlodipine, valsartan, and hydrochlorothiazide peaks of *Sample solution A*, *Sample solution B*, and *Sample solution C* correspond to those of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****Change to read:**• **PROCEDURE**

Use amber glassware for all solutions containing drug substances.

**Solution A:** Acetonitrile, water, and phosphoric acid (50:950:1)

**Solution B:** Acetonitrile, water, and phosphoric acid (950:50:1)

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
3	50	50
6	40	60
10	5	95
10.1	95	5
15	95	5

**Diluent:** Acetonitrile and water (500:500)

**0.1% Phosphoric acid:** Water and phosphoric acid (1000:1)

**Standard solution:** 0.14 mg/mL of USP Amlodipine Besylate RS, 0.064 mg/mL of USP Valsartan RS, and 0.025 mg/mL of USP Hydrochlorothiazide RS in *Diluent*

**Sample stock solution:** Transfer NLT 10 Tablets into a suitable volumetric flask. Add 0.1% Phosphoric acid to 4% of the total volume to disperse the Tablets. Sonicate for 10 min. Add 4% of the total volume of acetonitrile, swirl to mix, and add 60% of the total volume of *Diluent*. Sonicate for 20 min. Dilute with *Diluent* to volume to obtain solutions of nominal concentrations stated in *Table 2*. Centrifuge, and use the clear supernatant.

Table 2

Tablet Strength Amlodipine/ Valsartan/ Hydrochlorothiazide (mg/mg/ mg)	Nominal Concentration of Amlodipine (mg/mL)	Nominal Concentration of Valsartan (mg/mL)	Nominal Concentration of Hydrochlorothiazide (mg/mL)
5/160/12.5	0.1	3.2	0.25
10/160/12.5	0.2	3.2	0.25
5/160/25	0.1	3.2	0.5
10/160/25	0.2	3.2	0.5
10/320/25	0.1	3.2	0.25

**Sample solution A:** Nominally equivalent to 0.1 mg/mL of amlodipine in *Diluent* from *Sample stock solution*

**Sample solution B:** Nominally equivalent to 0.064 mg/mL of valsartan in *Diluent* from *Sample stock solution*

**Sample solution C:** Nominally equivalent to 0.025 mg/mL of hydrochlorothiazide in *Diluent* from *Sample stock solution*



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector****Assay:** 225 nm**Identification test A:** Diode array, •UV 200–400

nm • (RB 1-Jun-2016)

**Column:** 4.6-mm × 15-cm; 3-μm packing L1**Column temperature:** 40°**Flow rate:** 1.5 mL/min**Injection volume:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0 for amlodipine, valsartan, and hydrochlorothiazide**Relative standard deviation:** NMT 2.0% for amlodipine, valsartan, and hydrochlorothiazide**Analysis****Samples:** *Standard solution*, *Sample solution A*, *Sample solution B*, and *Sample solution C*Calculate the percentage of the labeled amount of amlodipine (C<sub>20</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>5</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

 $r_u$  = peak response of amlodipine from *Sample solution A* $r_s$  = peak response of amlodipine from the *Standard solution* $C_s$  = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL) $C_u$  = nominal concentration of amlodipine in *Sample solution A* (mg/mL) $M_{r1}$  = molecular weight of amlodipine, 408.88 $M_{r2}$  = molecular weight of amlodipine besylate, 567.05Calculate the percentage of the labeled amount of valsartan (C<sub>24</sub>H<sub>29</sub>N<sub>5</sub>O<sub>3</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 $r_u$  = peak response of valsartan from *Sample solution B* $r_s$  = peak response of valsartan from the *Standard solution* $C_s$  = concentration of USP Valsartan RS in the *Standard solution* (mg/mL) $C_u$  = nominal concentration of valsartan in *Sample solution B* (mg/mL)Calculate the percentage of the labeled amount of hydrochlorothiazide (C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 $r_u$  = peak response of hydrochlorothiazide from *Sample solution C* $r_s$  = peak response of hydrochlorothiazide from the *Standard solution* $C_s$  = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL) $C_u$  = nominal concentration of hydrochlorothiazide in *Sample solution C* (mg/mL)**Acceptance criteria:** •92.5%–107.5% • (RB 1-Jun-2016)**PERFORMANCE TESTS****Change to read:**• **DISSOLUTION (711)**• **Test 1** • (RB 1-Jun-2016)**Buffer:** Dissolve 6.805 g of monobasic potassium phosphate and 0.896 g of sodium hydroxide in

1000 mL of water. Adjust with 0.2 N sodium hydroxide or 1 M phosphoric acid to a pH of 6.8.

**Medium:** *Buffer*; 900 mL**Apparatus 2:** 50 rpm for 5/160/12.5, 10/160/12.5, 5/160/25, and 10/160/25 (mg/mg/mg) of Tablet strengths (amlodipine/valsartan/hydrochlorothiazide); 55 rpm for 10/320/25 (mg/mg/mg) of Tablet strengths (amlodipine/valsartan/hydrochlorothiazide)**Time:** 30 min**Solution A:** Acetonitrile, water, and phosphoric acid (50:950:1)**Solution B:** Acetonitrile, water, and phosphoric acid (950:50:1)**Mobile phase:** See *Table 3*.**Table 3**

Time (min)	Solution A (%)	Solution B (%)
0.00	67	33
2.50	23	77
2.51	67	33
4.00	67	33

**Diluent:** 1 mg/mL of polysorbate 80 in *Buffer***Standard stock solution A:** 0.07 mg/mL of USPAmlodipine Besylate and 0.124 mg/mL of USP Hydrochlorothiazide RS. Initially dissolve with 4% of the total volume of methanol, and dilute with *Diluent* to volume.**Standard stock solution B:** 3.2 mg/mL of USP Valsartan RS in methanol**Standard solution:** 0.014 mg/mL of USP Amlodipine Besylate RS, 0.16 mg/mL of USP Valsartan RS, and 0.0248 mg/mL of USP Hydrochlorothiazide RS in *Diluent* from *Standard stock solution A* and *Standard stock solution B*, respectively**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size. Discard at least the first 10 mL of the filtrate.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 250 nm**Column:** 4.6-mm × 5-cm; 3-μm packing L1**Column temperature:** 30°**Flow rate:** 1.5 mL/min**Injection volume:** 5 μL for 10/320/25 (mg/mg/mg) of Tablet strengths (amlodipine/valsartan/hydrochlorothiazide); 10 μL for 5/160/12.5, 10/160/12.5, 5/160/25, and 10/160/25 (mg/mg/mg) of Tablet strengths (amlodipine/valsartan/hydrochlorothiazide)**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 3.0 between amlodipine and valsartan**Tailing factor:** NMT 2.0 for amlodipine, valsartan, and hydrochlorothiazide**Relative standard deviation:** NMT 2.0% for amlodipine, valsartan, and hydrochlorothiazide**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of amlodipine (C<sub>20</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>5</sub>) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times (M_{r1}/M_{r2}) \times (1/L_i) \times 100$$

 $r_u$  = peak response of amlodipine from the *Sample solution* $r_s$  = peak response of amlodipine from the *Standard solution* $C_s$  = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL) $V$  = volume of *Medium*, 900 mL



$M_{r1}$  = molecular weight of amlodipine, 408.88  
 $M_{r2}$  = molecular weight of amlodipine besylate, 567.05  
 $L_1$  = label claim for amlodipine (mg/Tablet)  
 Calculate the percentage of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_2) \times 100$$

$r_U$  = peak response of valsartan from the *Sample solution*  
 $r_S$  = peak response of valsartan from the *Standard solution*  
 $C_S$  = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 900 mL  
 $L_2$  = label claim for valsartan (mg/Tablet)  
 Calculate the percentage of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_3) \times 100$$

$r_U$  = peak response of hydrochlorothiazide from the *Sample solution*  
 $r_S$  = peak response of hydrochlorothiazide from the *Standard solution*  
 $C_S$  = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 900 mL  
 $L_3$  = label claim for hydrochlorothiazide (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) is dissolved, NLT 80% (Q) of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) is dissolved, and NLT 80% (Q) of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium:** Proceed as directed under *Dissolution Test 1*, 900 mL.

#### Apparatus 2

For Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 5/160/12.5, 10/160/12.5, 5/160/25, 10/160/25, and 5/80/12.5 (mg/mg): 50 rpm

For Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 10/320/25 (mg/mg): 55 rpm

**Time:** 30 min for valsartan and hydrochlorothiazide, 45 min for amlodipine

**Buffer:** Mix 7.0 mL of triethylamine with 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.

**Solution A:** Acetonitrile and *Buffer* (10:90).

**Solution B:** Acetonitrile and *Buffer* (90:10).

**Mobile phase:** See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	90	10
7	30	70
8	90	10
15	90	10

**Standard stock solution A:** 0.35 mg/mL of USP Amlodipine Besylate RS prepared as follows. Initially dissolve in 10% of the final volume of methanol and dilute with *Medium* to volume.

**Standard stock solution B:** 1.6 mg/mL of USP Valsartan RS in methanol

**Standard stock solution C:** 0.7 mg/mL of USP Hydrochlorothiazide RS prepared as follows. Initially dissolve

in 25% of the final volume of methanol and dilute with *Medium* to volume.

**Standard solution:** ( $L_1/1000$ ) mg/mL of amlodipine, ( $L_2/1000$ ) mg/mL of valsartan, and ( $L_3/1000$ ) mg/mL of hydrochlorothiazide in *Diluent* from *Standard stock solution A*, *Standard stock solution B*, and *Standard stock solution C*, where  $L_1$  is the label claim of amlodipine in mg/Tablet,  $L_2$  is the label claim of valsartan in mg/Tablet, and  $L_3$  is the label claim of hydrochlorothiazide in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 1- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 237 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Temperatures**

**Autosampler:** 10°

**Column:** 50°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for each peak

**Relative standard deviation:** NMT 2.0% for each peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L_1) \times 100$$

$r_U$  = peak response of amlodipine from the *Sample solution*  
 $r_S$  = peak response of amlodipine from the *Standard solution*  
 $C_S$  = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 900 mL  
 $M_{r1}$  = molecular weight of amlodipine, 408.88  
 $M_{r2}$  = molecular weight of amlodipine besylate, 567.05  
 $L_1$  = label claim for amlodipine (mg/Tablet)  
 Calculate the percentage of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_2) \times 100$$

$r_U$  = peak response of valsartan from the *Sample solution*  
 $r_S$  = peak response of valsartan from the *Standard solution*  
 $C_S$  = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 900 mL  
 $L_2$  = label claim for valsartan (mg/Tablet)

Calculate the percentage of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_3) \times 100$$

$r_U$  = peak response of hydrochlorothiazide from the *Sample solution*  
 $r_S$  = peak response of hydrochlorothiazide from the *Standard solution*  
 $C_S$  = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 900 mL  
 $L_3$  = label claim for hydrochlorothiazide (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) is dissolved, NLT 80% (Q)



of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) is dissolved, and NLT 80% (Q) of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) is dissolved.

**Test 3:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 3.

**Medium:** Dissolve 6.80 g of monobasic potassium phosphate in 1000 mL of water. Adjust with 10% sodium hydroxide solution to a pH of 6.8; 1000 mL for valsartan and hydrochlorothiazide; 900 mL for amlodipine.

**Apparatus 2:** 50 rpm for valsartan and hydrochlorothiazide; 55 rpm for amlodipine in Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 10/320/25 (mg/mg/mg); and 50 rpm for amlodipine in Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 5/160/12.5, 10/160/12.5, 5/160/25, 10/160/25, and 5/80/12.5 (mg/mg/mg).

**Times:** 30 min for valsartan and hydrochlorothiazide, 45 min for amlodipine.

**Solution A:** Acetonitrile, trifluoroacetic acid and water (10:0.1:90).

**Solution B:** Acetonitrile, trifluoroacetic acid and water (90:0.1:10).

**Mobile phase:** See Table 5.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0.01	90	10
2.5	10	90
3.0	90	10
5.0	90	10

**Diluent:** Acetonitrile and water (50:50).

**Standard stock solution A:** 0.15 mg/mL of USP Amlodipine Besylate RS in *Medium* prepared as follows. Initially dissolve and sonicate in 5% of the final volume of *Diluent* and dilute with *Medium* to volume.

**Standard stock solution B:** 1.6 mg/mL of USP Valsartan RS in *Medium* prepared as follows. Initially dissolve and sonicate in 20% of the final volume of *Diluent* and dilute with *Medium* to volume.

**Standard stock solution C:** 0.25 mg/mL of USP Hydrochlorothiazide RS in *Medium* prepared as follows. Initially dissolve and sonicate in 10% of the final volume of *Diluent* and dilute with *Medium* to volume.

**Standard solution:** ( $L_1/1000$ ) mg/mL of amlodipine, ( $L_2/1000$ ) mg/mL of valsartan, and ( $L_3/1000$ ) mg/mL of hydrochlorothiazide in *Diluent* from *Standard stock solution A*, *Standard stock solution B*, and *Standard stock solution C*, where  $L_1$  is the label claim of amlodipine in mg/Tablet,  $L_2$  is the label claim of Valsartan in mg/Tablet, and  $L_3$  is the label claim of hydrochlorothiazide in mg/Tablet.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size. Discard at least the first few mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 237 for amlodipine and UV 270 nm for valsartan and hydrochlorothiazide.

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0 for each peak

**Relative standard deviation:** NMT 2.0% for each peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*. Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times (1/L_1) \times 100$$

$r_U$  = peak response of amlodipine from the *Sample solution*

$r_S$  = peak response of amlodipine from the *Standard solution*

$C_S$  = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$M_{r1}$  = molecular weight of amlodipine, 408.88

$M_{r2}$  = molecular weight of amlodipine besylate, 567.05

$L_1$  = label claim for amlodipine (mg/Tablet)

Calculate the percentage of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_2) \times 100$$

$r_U$  = peak response of valsartan from the *Sample solution*

$r_S$  = peak response of valsartan from the *Standard solution*

$C_S$  = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 1000 mL

$L_2$  = label claim for valsartan (mg/Tablet)

Calculate the percentage of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L_3) \times 100$$

$r_U$  = peak response of hydrochlorothiazide from the *Sample solution*

$r_S$  = peak response of hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 1000 mL

$L_3$  = label claim for hydrochlorothiazide (mg/Tablet)

#### Tolerances

**For Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 5/160/12.5, 10/160/12.5, 5/160/25, and 10/160/25 (mg/mg/mg):** NLT 75% (Q) of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) is dissolved, NLT 80% (Q) of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) is dissolved, and NLT 80% (Q) of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) is dissolved.

**For Tablets labeled to contain amlodipine/valsartan/hydrochlorothiazide, 5/160/25, and 10/320/25 (mg/mg/mg):** NLT 70% (Q) of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) is dissolved, NLT 80% (Q) of the labeled amount of valsartan ( $C_{24}H_{29}N_5O_3$ ) is dissolved, and NLT 80% (Q) of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) is dissolved. (RB 1-Jun-2016)

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### Change to read:

##### • ORGANIC IMPURITIES

Use amber glassware for all solutions containing drug substances.

**Mobile phase, Diluent, Sample solution A, Sample solution B, Sample solution C, and Chromatographic system:** Proceed as directed in the *Assay*.



**System suitability solution:** 0.02 mg/mL each of USP Benzothiadiazine Related Compound A RS and USP Valsartan Related Compound B RS, 0.005 mg/mL of USP Amlodipine Related Compound A RS, 0.14 mg/mL of USP Amlodipine Besylate RS, 0.064 mg/mL of USP Valsartan RS, and 0.025 mg/mL of USP Hydrochlorothiazide RS in *Diluent*

**Sensitivity solution:** 0.14 µg/mL of USP Amlodipine Besylate RS, 0.064 µg/mL of USP Valsartan RS, and 0.025 µg/mL of USP Hydrochlorothiazide RS in *Diluent*

**Standard solution:** 0.0005 mg/mL of USP Amlodipine Related Compound A RS, 0.0001 mg/mL of USP Benzothiadiazine Related Compound A RS, 0.0003 mg/mL of USP Amlodipine Besylate RS, 0.00015 mg/mL of USP Valsartan RS, and 0.0005 mg/mL of USP Hydrochlorothiazide RS in *Diluent*

#### System suitability

**Samples:** *System suitability solution, Sensitivity solution, and Standard solution*

#### Suitability requirements

**Signal-to-noise ratio:** NLT 10 for amlodipine, valsartan, and hydrochlorothiazide, *Sensitivity solution*

**Resolution:** NLT 2.0 between any adjacent peaks of benzothiadiazine related compound A, hydrochlorothiazide, amlodipine related compound A, amlodipine, valsartan related compound B, and valsartan, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for amlodipine related compound A, benzothiadiazine related compound A, amlodipine, valsartan, and hydrochlorothiazide, *Standard solution*

#### Analysis

**Samples:** *Sample solution A, Sample solution B, Sample solution C, and Standard solution*

Calculate the percentage of amlodipine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $r_U$  = peak response of amlodipine related compound A from *Sample solution A*  
 $r_S$  = peak response of amlodipine related compound A from the *Standard solution*  
 $C_S$  = concentration of USP Amlodipine Related Compound A RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of amlodipine in *Sample solution A* (mg/mL)  
 $M_{r1}$  = molecular weight of amlodipine related compound A free base, 406.86  
 $M_{r2}$  = molecular weight of amlodipine related compound A fumarate, 522.93

Calculate the percentage of any valsartan related degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of any valsartan related degradation product from *Sample solution B*  
 $r_S$  = peak response of valsartan from the *Standard solution*  
 $C_S$  = concentration of USP Valsartan RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of valsartan in *Sample solution B* (mg/mL)

Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of benzothiadiazine related compound A from *Sample solution C*  
 $r_S$  = peak response of benzothiadiazine related compound A from the *Standard solution*

$C_S$  = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydrochlorothiazide in *Sample solution C* (mg/mL)

Calculate the percentage of <sup>a</sup>chlorothiazide and hydrochlorothiazide dimer<sup>c</sup> (RB 1-Jun-2016) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of <sup>a</sup>chlorothiazide or hydrochlorothiazide dimer<sup>c</sup> (RB 1-Jun-2016) from *Sample solution C*  
 $r_S$  = peak response of hydrochlorothiazide from the *Standard solution*  
 $C_S$  = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of hydrochlorothiazide in *Sample solution C* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $r_U$  = peak response of each unspecified degradation product from *Sample solution A*  
 $r_S$  = peak response of amlodipine from the *Standard solution*  
 $C_S$  = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of amlodipine in *Sample solution A* (mg/mL)  
 $M_{r1}$  = molecular weight of amlodipine, 408.88  
 $M_{r2}$  = molecular weight of amlodipine besylate, 567.05

**Acceptance criteria:** See <sup>a</sup>Table 6<sup>a</sup> (RB 1-Jun-2016) Disregard amlodipine ethyl analog peak, valsartan related compound B peak, and any peaks below 0.1%.

<sup>a</sup>Table 6<sup>a</sup> (RB 1-Jun-2016)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A <sup>a</sup>	0.60	<sup>a</sup> 1.0 <sup>a</sup> (RB 1-Jun-2016)
<sup>a</sup> Chlorothiazide <sup>b</sup> (RB 1-Jun-2016) <sup>b</sup>	0.62	<sup>a</sup> 0.50 <sup>a</sup> (RB 1-Jun-2016)
Hydrochlorothiazide	0.64	—
Devaleryl valsartan <sup>c</sup>	0.71	0.2
<sup>a</sup> Hydrochlorothiazide dimer <sup>c</sup> (RB 1-Jun-2016) <sup>d</sup>	0.89	<sup>a</sup> 0.50 <sup>a</sup> (RB 1-Jun-2016)
Amlodipine related compound A <sup>e</sup>	0.96	0.5
Amlodipine	1.00	—

<sup>a</sup> 4-Amino-6-chloro-1,3-benzenedisulfonamide.

<sup>b</sup> 6-Chloro-2 H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. (RB 1-Jun-2016)

<sup>c</sup> N-[(2'-(1H-Tetrazole-5-yl)biphenyl-4-yl)methyl]-L-valine.

<sup>d</sup> 6-Chloro-N-[(6-chloro-7-sulfamoyl-2,3-dihydro-4H-1,2,4-benzothiadiazine-4-yl 1,1-dioxide)methyl]3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. (RB 1-Jun-2016)

<sup>e</sup> 3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].

<sup>f</sup> These are specified unidentified degradation products. No information is available about chemical structures or chemical names for these impurities.

<sup>g</sup> Diethyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. <sup>h</sup> Process related impurity given for information only. (RB 1-Jun-2016)

<sup>h</sup> (S)-N-Butyryl-N-[(2'-(1H-tetrazole-5-yl)biphenyl-4-yl)-methyl]-valine. <sup>i</sup> Process related impurity given for information only. (RB 1-Jun-2016)

<sup>i</sup> Benzenesulfonic acid is the counter ion to the amlodipine, and peaks at RRT of 0.33 and 0.42 are not considered as degradation products.



Table 60 (RB 1-Jun-2016) (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Valsartan related degradation product 1 <sup>i</sup>	1.04	0.2
Amlodipine ethyl analog <sup>a</sup>	1.08	—
Valsartan related compound B <sup>b</sup>	1.22	—
Valsartan related degradation product 2 <sup>i</sup>	1.27	0.2
Valsartan	1.36	—
Valsartan related degradation product 3 <sup>i</sup>	1.51	0.2
Valsartan related degradation product 4 <sup>i</sup>	1.62	0.2
Any other unspecified degradation product <sup>i</sup>	—	0.2
Total degradation products	—	2.0 (RB 1-Jun-2016)

<sup>a</sup> 4-Amino-6-chloro-1,3-benzenedisulfonamide.<sup>b</sup> 6-Chloro-2-*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. (RB 1-Jun-2016)

Jun-2016)

<sup>c</sup> *N*-[2'-(1-*H*-Tetrazole-5-yl)biphenyl-4-yl]methyl-L-valine.<sup>d</sup> 6-Chloro-*N*-[(6-chloro-7-sulfamoyl-2,3-dihydro-4*H*-1,2,4-benzothiadiazine-4-yl 1,1-dioxide)methyl]3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide. (RB 1-Jun-2016)<sup>e</sup> 3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate].<sup>f</sup> These are specified unidentified degradation products. No information is available about chemical structures or chemical names for these impurities.<sup>g</sup> Diethyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. Process related impurity given for information only. (RB 1-Jun-2016)<sup>h</sup> (5)-*N*-Butyryl-*N*-[2'-(1-*H*-tetrazole-5-yl)biphenyl-4-yl]methyl-valine.<sup>i</sup> Process related impurity given for information only. (RB 1-Jun-2016)<sup>j</sup> Benzenesulfonic acid is the counter ion to the amlodipine, and peaks at RRT of 0.33 and 0.42 are not considered as degradation products.**ADDITIONAL REQUIREMENTS**

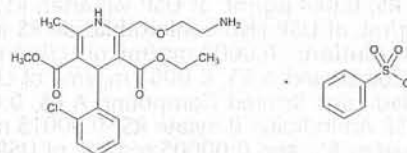
- **PACKAGING AND STORAGE:** Store at controlled room temperature in tight containers in a dry place.

**Add the following:**

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. (RB 1-Jun-2016)
- **USP REFERENCE STANDARDS (11)**
  - USP Amlodipine Besylate RS
  - USP Amlodipine Related Compound A RS
  - 3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.
  - $C_{20}H_{23}ClN_2O_5 \cdot C_4H_4O_4$  522.93
  - USP Benzothiadiazine Related Compound A RS
  - 4-Amino-6-chloro-1,3-benzenedisulfonamide.
  - $C_6H_8ClN_3O_4S_2$  285.73

USP Hydrochlorothiazide RS

USP Valsartan RS

**Amlodipine Besylate** $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$  567.05

3,5-Pyridinedicarboxylic acid, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-, 3-ethyl 5-methyl ester, (±)-, monobenzenesulfonate.

3-Ethyl 5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulfonate [111470-99-6].

Monohydrate 585.07

» Amlodipine Besylate is anhydrous or hydrated and contains not less than 97.0 percent and not more than 102.0 percent of  $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers, protected from light. Store at room temperature.

**USP Reference standards (11)**—

USP Amlodipine Besylate RS

**Labeling**—Where it is the hydrated form, the label so indicates.

**Identification**—*A: Infrared Absorption* (197M).

*B:* The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Optical rotation** (781A): between  $-0.10^\circ$  and  $+0.10^\circ$ , measured at  $20^\circ$ .

*Test solution:* 10 mg per mL, in methanol.

**Water Determination, Method I** (921): not more than 0.5% for the anhydrous form. If labeled as the hydrated form, the limit is between 3.1% and 5.0%.

**Residue on ignition** (281): not more than 0.2%.

**Delete the following:**

• **Heavy metals, Method II** (231): 0.002%. (Official 1-Jan-2018)

**Related compounds**—

TEST 1—

*Adsorbent:* 0.25-mm layer of chromatographic silica gel mixture.

*Test solution*—Transfer 140 mg of Amlodipine Besylate to a 2-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix.

*System suitability solution*—Transfer about 14 mg of USP Amlodipine Besylate RS to a suitable container, dissolve in 0.2 mL of methanol, and mix.

*Standard stock solution*—Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in methanol to obtain a solution containing 7.0 mg per mL.

*Standard solution 1*—Transfer 3.0 mL of the *Standard stock solution* to a 100-mL volumetric flask, dilute with methanol to volume, and mix.



**Standard solution 2**—Transfer 1.0 mL of the *Standard solution* to another 100-mL volumetric flask, dilute with methanol to volume, and mix.

**Application volume:** 10  $\mu$ L.

**Developing solvent system**—Use the upper layer of a mixture of methyl isobutyl ketone, water, and glacial acetic acid (50:25:25).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Dry the plate for 15 minutes at 80°. Examine the plate under UV light at 254 nm and 365 nm. The chromatogram from the *System suitability solution* shows two clearly separated minor spots with  $R_f$  values of about 0.18 and 0.22. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with those of the principal spots in the chromatograms of the *Standard solutions*. Any spot obtained from the *Test solution*, except for the principal spot, is not greater in size than the spot obtained from *Standard solution 1* (0.3%), and at most two spots are more intense than the spot obtained from *Standard solution 2* (0.1%).

#### TEST 2—

**pH 3.0 Buffer and Mobile phase**—Prepare as directed in the *Assay*.

**System suitability solution**—Dissolve about 5 mg of Amlodipine Besylate in 5 mL of hydrogen peroxide, and heat at 70° for 45 minutes.

**Standard solution**—Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.003 mg per mL.

**Test solution**—Transfer about 50 mg of Amlodipine Besylate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between amlodipine impurity A and amlodipine is not less than 4.5. [NOTE—For the purpose of identification, the relative retention times are about 0.2 for benzene sulfonate, 0.5 for amlodipine impurity A, and 1.0 for amlodipine. Amlodipine impurity A is 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methylpyridine-3,5-dicarboxylate.] Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the standard deviation for replicate injections is not more than 10.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for a period of time that is about 3 times the retention time of amlodipine, and measure the peak responses. Calculate the percentage of each impurity in the portion of Amlodipine Besylate taken by the formula:

$$100(1/F)(C_S/C_T)(r_i/r_s)$$

in which  $F$  is the relative response factor, which is equal to 0.5 for amlodipine impurity A and to 1.0 for other impurities;  $C_S$  and  $C_T$  are the concentrations, in mg per mL, of amlodipine besylate in the *Standard solution* and the *Test solution*, respectively;  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_s$  is the peak response for amlodipine besylate obtained from the *Standard solution*: not more than 0.3% of amlodipine impurity A is found, and not more than 0.3% of total other impurities is found. Disregard any peak less than 0.03%, and disregard any peak due to benzene sulfonate.

#### Assay—

**pH 3.0 Buffer**—Dissolve 7.0 mL of triethylamine in 800 mL of water. Adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ , and dilute with water to 1 L.

**Mobile phase**—Prepare a filtered and degassed mixture of pH 3.0 Buffer, methanol, and acetonitrile (50:35:15). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Amlodipine Besylate RS in *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

**Assay preparation**—Transfer about 50 mg of Amlodipine Besylate, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 237-nm detector and a 3.9-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of  $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$  in the portion of Amlodipine Besylate taken by the formula:

$$100(C_S/C_U)(r_U/r_S)$$

in which  $C_S$  and  $C_U$  are the concentrations, in mg per mL, of amlodipine besylate in the *Standard preparation* and the *Assay preparation*, respectively; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Amlodipine Besylate Tablets

### DEFINITION

Amlodipine Besylate Tablets contain NLT 90% and NMT 110% of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ).

### IDENTIFICATION

#### • A. ULTRAVIOLET ABSORPTION (197U)

**Standard solution and Sample solution:** Prepare as directed in the test for *Dissolution*.

**Acceptance criteria:** Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** Add 7.0 mL of triethylamine into a 1000-mL flask containing 900 mL of water. Adjust the solution with phosphoric acid to a pH of  $3.0 \pm 0.1$ . Dilute with water to volume, and mix well.

**Mobile phase:** Methanol, acetonitrile, and Buffer (35:15:50)

**Standard solution:** 0.0275 mg/mL of USP Amlodipine Besylate RS and 0.0025 mg/mL of USP Amlodipine Related Compound A RS in *Mobile phase*

**Sample solution:** Nominally 0.02 mg/mL of amlodipine in *Mobile phase* prepared as follows. Place NLT 5 Tablets in a suitable volumetric flask, and add sufficient quantity of *Mobile phase* to disintegrate the Tablets. Shake for 30 min, and dilute with *Mobile phase* to volume. Pass the sample through a syringe tip filter of 0.45- $\mu$ m pore size. Discard the first few mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 237 nm  
 Column: 3.9-mm × 15-cm; 5-μm packing L1  
 Flow rate: 1 mL/min  
 Injection volume: 50 μL

Run time: NLT 3 times the retention of the amlodipine peak

#### System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for amlodipine and amlodipine related compound A are about 1.0 and 0.5 respectively.]

#### Suitability requirements

Resolution: NLT 8.5 between amlodipine and amlodipine related compound A

Tailing factor: NMT 2.0 for both amlodipine and amlodipine related compound A

Relative standard deviation: NMT 5.0% for amlodipine related compound A

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amlodipine Besylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of amlodipine, 408.88

$M_{r2}$  = molecular weight of amlodipine besylate, 567.05

Acceptance criteria: 90%–110% of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ )

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

[NOTE—Do not expose any of the solutions to stainless steel because of the degradation of amlodipine.]

Medium: 0.01 N hydrochloric acid; 500 mL

Apparatus 2: 75 rpm. [NOTE—Use paddles covered with Teflon or made of any inert material except stainless steel.]

Time: 30 min

Standard solution: Make appropriate dilutions of USP Amlodipine Besylate RS in *Medium* to obtain the following concentrations: 0.00695 mg/mL for Tablets labeled to contain 2.5 mg, 0.0139 mg/mL for Tablets labeled to contain 5 mg, and 0.0278 mg/mL for Tablets labeled to contain 10 mg. These solutions are stable for 1 day.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

#### Analysis

Samples: *Standard solution* and *Sample solution*

Determine the amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) dissolved by using UV absorption at the wavelength of maximum absorbance at about 239 nm on portions of the *Sample solution* in comparison with the *Standard solution*, using a 1-cm quartz cell and the *Medium* as the blank.

Calculate the percentage of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$D$  = dilution factor of the *Sample solution*

$M_{r1}$  = molecular weight of amlodipine, 408.88

$M_{r2}$  = molecular weight of amlodipine besylate, 567.05

$V$  = volume of *Medium*, 500 mL

$L$  = label claim (mg/Tablet)

Tolerances: NLT 75% ( $Q$ ) of the labeled amount of amlodipine ( $C_{20}H_{25}ClN_2O_5$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

Buffer, Mobile phase, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

Sample solution: Place a suitable number of Tablets into a 25-mL volumetric flask to obtain a solution with a final nominal concentration of 0.4 mg/mL of amlodipine. Add about 10 mL of *Mobile phase* to the flask. Swirl to disintegrate the Tablets, then sonicate for 5 min to completely dissolve, and cool the sample to room temperature. Dilute with *Mobile phase* to volume. Stir for an additional 15 min using a magnetic stir bar, and pass the sample through a syringe tip filter of 0.45-μm pore size, discarding the first 5 mL.

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amlodipine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of amlodipine related compound A from the *Sample solution*

$r_S$  = peak response of amlodipine related compound A from the *Standard solution*

$C_S$  = concentration of USP Amlodipine Related Compound A RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of amlodipine related compound A, 406.86

$M_{r2}$  = molecular weight of amlodipine related compound A fumarate, 522.93

Calculate the percentage of amlodipine glucose/galactose adduct or amlodipine lactose adduct, if present, and any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of the amlodipine glucose/galactose adduct, amlodipine lactose adduct, or any unspecified degradation product from the *Sample solution*

$r_S$  = peak response of amlodipine from the *Standard solution*

$C_S$  = concentration of USP Amlodipine Besylate RS from the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amlodipine in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of amlodipine, 408.9

$M_{r2}$  = molecular weight of amlodipine besylate, 567.05

Acceptance criteria: See *Table 1*.



Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amlodipine related compound A <sup>a</sup>	0.50	1.0
Amlodipine lactose adduct <sup>b</sup>	0.80	0.5
Amlodipine glucose/galactose adduct <sup>b</sup>	0.90	0.5
Amlodipine besylate	1.0	—
Any unspecified degradation product	—	0.2

<sup>a</sup> 3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.

<sup>b</sup> Formulation-specific impurities.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Amlodipine Besylate RS  
USP Amlodipine Related Compound A RS  
3-Ethyl, 5-methyl [2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-3,5-pyridinedicarboxylate] fumarate.  
 $C_{20}H_{23}ClN_2O_5 \cdot C_4H_4O_4$  522.93

## Aromatic Ammonia Spirit

### DEFINITION

Aromatic Ammonia Spirit is a hydroalcoholic solution that contains, in each 100 mL, NLT 1.7 g and NMT 2.1 g of total ammonia (NH<sub>3</sub>) and Ammonium Carbonate corresponding to NLT 3.5 g and NMT 4.5 g of ammonium carbonate [(NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>].

### ASSAY

#### • TOTAL AMMONIA (NH<sub>3</sub>)

Sample: 10.0 mL

Titrimetric system

(See *Titrimetry* (541), *Residual Titrations*.)

Mode: Residual titration

Titrant: 0.5 N sodium hydroxide VS

**Analysis:** Transfer the *Sample* to a 250-mL conical flask containing 50 mL of water. Add 30.0 mL of 0.5 N sulfuric acid VS, and boil until the solution becomes clear. Cool, add methyl red TS, and titrate the excess acid with *Titrant*. Perform a blank determination. Each mL of 0.5 N sulfuric acid is equivalent to 8.515 mg of ammonia (NH<sub>3</sub>).

#### • AMMONIUM CARBONATE

Sample: 10.0 mL

Titrimetric system

(See *Titrimetry* (541), *Residual Titrations*.)

Mode: Residual titration

Titrant: 0.5 N sulfuric acid VS

**Analysis:** Transfer the *Sample* to a 300-mL flask. Add 30 mL of 0.5 N sodium hydroxide, and boil the mixture, replacing the water lost by evaporation, until the vapors no longer turn moistened red litmus paper blue. Cool, dilute with 100 mL of cold, carbon dioxide-free water, add 6 drops of phenolphthalein TS, then add just enough 0.5 N sulfuric acid VS to discharge the color of the phenolphthalein. Add methyl orange TS, and titrate with *Titrant*. Perform a blank determination. Each mL of *Titrant* consumed in the titration with methyl orange TS

is equivalent to 48.04 mg of ammonium carbonate [(NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>].

**Acceptance criteria:** In each 100 mL, NLT 1.7 g and NMT 2.1 g of total ammonia (NH<sub>3</sub>) and Ammonium Carbonate corresponding to NLT 3.5 g and NMT 4.5 g of ammonium carbonate [(NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>].

### OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method I (611):** 62.0%–68.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature not exceeding 30°.

## Ammonium Chloride

NH<sub>4</sub>Cl 53.49

Ammonium chloride.

Ammonium chloride [12125-02-9].

» Ammonium Chloride contains not less than 99.5 percent and not more than 100.5 percent of NH<sub>4</sub>Cl, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Identification**—A solution (1 in 10) responds to the tests for Ammonium (191) and for Chloride (191).

**pH** (791): between 4.6 and 6.0, in a solution (1 in 20).

**Loss on drying** (731)—Dry it over silica gel for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281)—Add 1 mL of sulfuric acid to about 2 g, accurately weighed, and heat the mixture gently until volatilization is complete: the residue is white, and when ignited, not more than 0.1% of nonvolatile substance remains.

**Limit of thiocyanate**—Acidify 10 mL of a solution (1 in 10) with hydrochloric acid, and add a few drops of ferric chloride TS: no orange-red color is produced.

### Delete the following:

• **Heavy metals, Method I (231):** 0.001%. • (Official 1-Jan-2018)

**Assay**—Transfer about 100 mg of Ammonium Chloride, accurately weighed, to a conical flask, add 10 mL of water, and swirl to dissolve. Add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 5.349 mg of NH<sub>4</sub>Cl.

## Ammonium Chloride Injection

» Ammonium Chloride Injection is a sterile solution of Ammonium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of NH<sub>4</sub>Cl. Hydrochloric acid may be added to adjust the pH.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

**Labeling**—The label states the content of ammonium chloride in terms of weight and of milliequivalents in a given volume. The label states also the total osmolar concentration in mOsmol per L or per mL. The label states that the



Injection is not for direct injection but is to be diluted with Sodium Chloride Injection to the appropriate strength before use.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Identification**—It responds to the tests for *Ammonium* (191) and for *Chloride* (191).

**Bacterial Endotoxins Test** (85)—It contains not more than 1.72 USP Endotoxin Units per mEq of chloride.

**pH** (791): between 4.0 and 6.0, in a concentration of not more than 100 mg of ammonium chloride per mL.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Chloride content**—Transfer an accurately measured volume of Injection, evaporated, if necessary, equivalent to about 2 g of ammonium chloride, to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a conical flask, add 10 mL of glacial acetic acid, 75 mL of methanol, and 0.5 mL of eosin Y TS. Titrate, with shaking, with 0.1 N silver nitrate VS to a pink endpoint. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. The content of Cl is between 63.0% and 70.3% of the labeled amount of ammonium chloride.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 200 mg of ammonium chloride, to a 500-mL Kjeldahl flask, dilute with water to 200 mL, mix, and add 50 mL of sodium hydroxide solution (2 in 5). Immediately connect the flask by means of a distillation trap to a well-cooled condenser, the delivery tube of which dips into 40 mL of boric acid solution (1 in 25) contained in a suitable receiver. Heat to boiling, and distill about 200 mL. Cool the liquid in the receiver, if necessary, then add methyl red TS, and titrate with 0.1 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sulfuric acid is equivalent to 5.349 mg of  $\text{NH}_4\text{Cl}$ .

## Ammonium Chloride Delayed-Release Tablets

» Ammonium Chloride Delayed-Release Tablets contain not less than 94.0 percent and not more than 106.0 percent of the labeled amount of  $\text{NH}_4\text{Cl}$ . Ammonium Chloride Delayed-Release Tablets are enteric-coated.

**Packaging and storage**—Preserve in tight containers.

**Identification**—A filtered solution of finely powdered Tablets, equivalent to ammonium chloride solution (1 in 10), responds to the tests for *Ammonium* (191) and for *Chloride* (191).

**Disintegration** (701): 2 hours, determined as directed for *Enteric-Coated Tablets*.

**Limit of thiocyanate**—Powder and dissolve in water a sufficient number of Tablets to make about 25 mL of ammonium chloride solution (1 in 10), and filter. Acidify 10 mL of the solution with hydrochloric acid, and add a few drops of ferric chloride TS: no reddish orange color is produced.

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 200 mg of ammonium chloride, to a 500-mL Kjeldahl flask, and add 200 mL of water and 50 mL of sodium hydroxide solution (2 in 5). Immediately connect the flask by means of a distillation trap to a well-cooled condenser, the delivery tube of which dips into 40 mL of

boric acid solution (1 in 25) contained in a suitable receiver. Heat to boiling, and distill about 200 mL. Cool the liquid in the receiver, if necessary, then add methyl red TS, and titrate with 0.1 N sulfuric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sulfuric acid is equivalent to 5.349 mg of  $\text{NH}_4\text{Cl}$ .

## Ferric Ammonium Citrate

» Ferric Ammonium Citrate contains not less than 16.5 percent and not more than 18.5 percent of iron (Fe).

**Packaging and storage**—Preserve in tight, light-resistant containers, in a cool place.

**Identification**—

A: Ignite about 0.5 g: it chars, and leaves a residue of iron oxide.

B: To 10 mL of a solution of Ferric Ammonium Citrate (1 in 100) add 6 N ammonium hydroxide dropwise: the solution darkens, but no precipitate forms.

C: To 5 mL of a solution of Ferric Ammonium Citrate (1 in 100) add 0.3 mL of potassium permanganate TS and 4 mL of mercuric sulfate TS, and heat the mixture to boiling: a white precipitate forms.

**Ferric citrate**—To a solution of Ferric Ammonium Citrate (1 in 100) add potassium ferrocyanide TS: no blue precipitate is formed.

**Sulfate** (221)—Dissolve 100 mg in 1 mL of 2.7 N hydrochloric acid, and dilute with water to 30 mL. Add 3 mL of barium chloride TS, dilute with water to 50 mL, and mix: any turbidity formed after 10 minutes is not greater than that produced in a similarly treated control solution containing 0.31 mL of 0.020 N sulfuric acid (0.3%).

**Oxalate**—Transfer 1 g to a 125-mL separator, dissolve in 10 mL of water, add 2 mL of hydrochloric acid, and extract successively with one 50-mL portion and one 20-mL portion of ether. Transfer the combined ether extracts to a 150-mL beaker, add 10 mL of water, and remove the ether by evaporation on a steam bath. Add 1 drop of glacial acetic acid and 1 mL of calcium acetate solution (1 in 20): no turbidity is produced within 5 minutes.

**Mercury**—

*Mercury Stock Solution and Standard Mercury Solution*—Proceed as directed for *Method I* under *Mercury* (261).

*Mercury Detection Instrument, Aeration Apparatus, and Stannous Chloride Solution*—Proceed as directed for *Method IIa* and *Method IIb* under *Mercury* (261).

**Standard solutions**—Transfer 0.25, 0.50, 1.0, and 3.5 mL of *Standard Mercury Solution* to four separate glass-stoppered bottles, such as biological oxygen-demand bottles, of about 300-mL capacity. Dilute the contents of each bottle with water to 100 mL, and mix. These solutions contain the equivalent of 2.5, 5.0, 10.0, and 35.0 ng of mercury per mL, respectively.

**Test solution**—Transfer about 1.000 g of Ferric Ammonium Citrate, accurately weighed, to a 200-mL centrifuge bottle with a polytetrafluoroethylene-lined screw cap, and add 5 mL of nitric acid and 5 mL of hydrochloric acid. Close the bottle tightly, digest on a steam bath for 1 hour, and cool. Quantitatively transfer the solution to a suitable glass-stoppered bottle, dilute with water to 100 mL, and bubble air through the solution for 2 minutes. Prepare a reagent blank in the same manner.

**Procedure**—Add 5 mL of *Stannous Chloride Solution* to each solution, and immediately insert the bubbler of the *Aeration Apparatus*. Obtain the absorbances as directed by the instrument manufacturer's operating instructions. Per-



form a blank determination, and make any necessary correction. Plot the absorbances of the *Standard solutions* versus concentrations, in  $\mu\text{g}$  per mL, of mercury, and draw the straight line best fitting the plotted points. From the graph so obtained, determine the concentration, in  $\mu\text{g}$  per g, of mercury in the *Test solution*: not more than 10  $\mu\text{g}$  per g is found.

#### Limit of lead—

**Standard stock solution**—Dissolve about 159.8 mg of lead nitrate, accurately weighed, in 100 mL of water containing 1 mL of nitric acid. Dilute with water to 1000.0 mL, and mix.

**Standard solution**—[NOTE—Prepare this solution on the day of use.] Transfer 10.0 mL of *Standard stock solution* to a 500-mL volumetric flask, dilute with water to volume, and mix. Each mL contains the equivalent of 2  $\mu\text{g}$  of lead (Pb).

**Test solution**—Transfer about 15 g of Ferric Ammonium Citrate, accurately weighed, to a 100-mL volumetric flask (previously rinsed with nitric acid and water), dissolve in a mixture of 50 mL of water and 1 mL of nitric acid, dilute with water to volume, and mix.

**Procedure**—Using a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a deuterium arc background corrector, a digital readout device, and a burner head capable of handling 15% solids content, perform a blank determination with water, following the manufacturer's operating instructions. Separately aspirate portions of the *Standard solution* and the *Test solution*, and record the absorbances. Calculate the lead content, in  $\mu\text{g}$  per g, in the portion of Ferric Ammonium Citrate taken by the formula:

$$100(C/W)(A_U/A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of lead in the *Standard solution*; W is the weight, in g, of Ferric Ammonium Citrate taken; and  $A_U$  and  $A_S$  are the absorbances of the *Test solution* and the *Standard solution*, respectively: not more than 10  $\mu\text{g}$  per g is found.

**Assay**—Transfer about 1 g of Ferric Ammonium Citrate, accurately weighed, to a 250-mL conical flask, and dissolve in 25 mL of water and 5 mL of hydrochloric acid. Add 4 g of potassium iodide, insert the stopper, and allow to stand protected from light for 15 minutes. Add 100 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, using starch TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of iron (Fe).

### Ferric Ammonium Citrate for Oral Solution

» Ferric Ammonium Citrate for Oral Solution contains Ferric Ammonium Citrate and an effervescent mixture of a suitable organic acid and an alkali metal bicarbonate. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of Fe. It may contain one or more suitable flavors, colors, or stabilizing agents.

**Packaging and storage**—Preserve in tight, light-resistant containers, and store in a cool place.

**Identification**—A 6-g portion dissolves in 600 mL of water with effervescence. The collected gas meets the requirements of the test for *Bicarbonate* (191), and the resulting solution meets the requirements of the tests for *Iron* (191) and for *Citrate* (191).

#### Uniformity of dosage units (905)—

FOR POWDER PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

#### Deliverable volume (698)—

FOR POWDER PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Assay**—Transfer about 6 g of Ferric Ammonium Citrate for Oral Solution, accurately weighed, to a 250-mL conical flask, and dissolve in 100 mL of water. Allow the gas to escape, add 5 mL of hydrochloric acid and 4 g of potassium iodide, insert the stopper, and allow to stand protected from light for 15 minutes. Add 25 mL of water, and titrate the liberated iodine with 0.1 N sodium thiosulfate VS, using starch TS as the indicator. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of Fe.

### Ammonium Molybdate

$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  1236.00  
Molybdate ( $\text{Mo}_7\text{O}_{24}^{6-}$ ), hexaammonium, tetrahydrate;  
Hexaammonium molybdate tetrahydrate [12054-85-2].

#### DEFINITION

Ammonium Molybdate contains NLT 99.3% and NMT 101.8% of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ .

#### IDENTIFICATION

##### • PROCEDURE

**Sample:** 0.6 g

**Analysis:** Dissolve the *Sample* in 1.4 mL of water and 1.45 mL of ammonium hydroxide. Cool this mixture, and add slowly, with mixing, 7.2 mL of a well-cooled mixture of 3.2 mL of nitric acid and 4 mL of water. Allow to stand for 24–48 h, and pass through a sintered-glass filter. To 5 mL of the filtrate add 2 mL of dibasic sodium phosphate TS.

**Acceptance criteria:** A yellow precipitate is formed, and it is soluble in an excess of 6 N ammonium hydroxide.

#### ASSAY

##### • PROCEDURE

**Sample solution:** Dissolve 0.7 g of Ammonium Molybdate in 100 mL water. Adjust with dilute nitric acid to a pH of 4.0. Add saturated hexamethylenetetramine solution to achieve a pH of 5–6.

**Analysis:** Heat the *Sample solution* to 60°, and add 0.2 mL of 0.1% 4-[2-pyridylazo]resorcinol solution in alcohol. Titrate with 0.1 M lead nitrate VS from the yellow color to the first permanent pink endpoint. Carry out a blank titration. Each mL of 0.1 M lead nitrate is equivalent to 17.66 mg of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ .

**Acceptance criteria:** 99.3%–101.8%

#### IMPURITIES

##### Inorganic Impurities

##### • ARSENATE, PHOSPHATE, AND SILICATE

**Sample solution:** Dissolve 2.5 g of the analyte in 70 mL of water in a container other than glass.

**Control solution:** Dissolve 0.5 g of the analyte in 70 mL of water in a container other than glass, and add an amount of sodium silicate solution equivalent to 0.02 mg of silica ( $\text{SiO}_2$ ).

##### Analysis

**Samples:** *Sample solution* and *Control solution*

Adjust with 1.2 N hydrochloric acid to a pH of between 3 and 4, transfer to a glass container, add 2 mL of bromine TS, and adjust with 1.2 N hydrochloric acid to a pH of  $1.8 \pm 0.1$ . Heat almost to boiling, and cool to room temperature. Dilute with



water to 90 mL, add 10 mL of hydrochloric acid, and transfer to a separator. Add 1 mL of butyl alcohol and 30 mL of 4-methyl-2-pentanone, shake vigorously, and allow the phases to separate. Discard the aqueous phase, and wash the ketone phase with three successive 10-mL portions of 1.2 N hydrochloric acid, discarding the washings. To the washed ketone phase add 10 mL of 1.2 N hydrochloric acid to which has just been added 0.2 mL of a freshly prepared solution (1 in 50) of stannous chloride in hydrochloric acid.

**Acceptance criteria:** Any blue color in the *Sample solution* does not exceed that in the *Control solution* (NMT 10 ppm).

- **CHLORIDE AND SULFATE, Chloride** (221): A 0.5-g portion shows no more chloride than 0.30 mL of 0.001 N hydrochloric acid (NMT 20 ppm).
- **CHLORIDE AND SULFATE, Sulfate** (221): A 0.25-g portion shows no more sulfate than corresponds to 1.0 mL of 0.001 N sulfuric acid (NMT 200 ppm).

#### Delete the following:

#### • HEAVY METALS (231)

**Sample stock solution:** Dissolve 2.0 g of Ammonium Molybdate in 20 mL of water, add 10 mL of 2.5 N sodium hydroxide and 2 mL of ammonium hydroxide, and dilute with water to 40 mL.

**Control solution:** To 10 mL of *Sample stock solution* add 1.0 mL of *Standard Lead Solution*, prepared as directed under *Heavy Metals* (231), and dilute with water to 40 mL.

**Sample solution:** Dilute the remaining 30-mL portion of the *Sample stock solution* with water to 40 mL.

**Analysis:** To both the *Sample solution* and the *Control solution* add 1.2 mL of thioacetamide–glycerin base TS and 2 mL of pH 3.5 Acetate Buffer, and allow to stand for 5 min.

**Acceptance criteria:** Any color in the *Sample solution* does not exceed that in the *Control solution* (NMT 10 ppm). • (Official 1-Jan-2018)

#### • INSOLUBLE SUBSTANCES

**Sample:** 20 g

**Analysis:** Dissolve the *Sample* in 200 mL of water in a beaker, heat to boiling, cover, and heat on a steam bath for 1 h. Pass the hot solution through a tared filtering crucible, wash the insoluble residue with hot water, and dry at 105° for 2 h.

**Acceptance criteria:** The weight of the residue is NMT 1 mg (0.005%).

#### • NITRATE

**Sample:** 1 g

**Analysis:** Dissolve the *Sample* in 10 mL of water containing 5 mg of sodium chloride, and add 0.10 mL of a solution (1 in 1000) of indigo carmine in 3.6 N sulfuric acid.

**Acceptance criteria:** The blue color is not completely discharged in 5 min.

#### • MAGNESIUM AND ALKALI SALTS

**Sample:** 5.0 g

**Analysis:** Dissolve the *Sample* in 50 mL of water, and filter. To the filtrate add 0.5 g of sodium carbonate and 25 mL of 2.5 N sodium hydroxide. Boil the solution gently for 5 min, cool, and pass through an ignited and tared filter. Wash the filter with 1 N ammonium hydroxide. Ignite the filter at 800 ± 25° for 30 min.

**Acceptance criteria:** The weight of the residue does not exceed 1 mg (NMT 0.02%).

#### • PHOSPHATE

**Standard solution:** Dissolve 143.3 mg of dried monobasic potassium phosphate in water to make 1000 mL, and then dilute 1.0 mL of this solution with 3 N ammonium hydroxide to 100 mL.

**Sample solution:** Dissolve 20 g of the analyte in 100 mL of 3 N ammonium hydroxide.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Add 3.5 mL of ferric nitrate solution (1 in 10), and allow to stand for 15 min. Warm gently to coagulate the precipitate, and filter. Wash the filter several times with 1.5 N ammonium hydroxide, then wash the filter with 60 mL of warm 4 N nitric acid to dissolve the residue on the filter, collecting the filtrate in a glass-stoppered, 250-mL conical flask. Add 13 mL of ammonium hydroxide, warm to 40°, add 50 mL of ammonium molybdate TS, shake for 5 min, and allow to stand at 40° for 2 h.

**Acceptance criteria:** Any yellow precipitate formed from the *Sample solution* does not exceed that from the *Standard solution* (5 ppm).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

### Ammonium Molybdate Injection

» Ammonium Molybdate Injection is a sterile solution of Ammonium Molybdate in Water for Injection. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of molybdenum (Mo).

**Packaging and storage—**Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

**Labeling—**Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.

#### Identification—

**A:** The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 313 nm when tested as directed for *Procedure* in the *Assay*.

**B:** Add 0.3 mL of alkaline mercuric-potassium iodide TS to 5 mL of Injection: a reddish-brown color develops.

**C:** Evaporate 50 mL of Injection on a steam bath to a volume of about 0.3 mL, and add 0.3 mL of ammonium hydroxide. Cool, and add slowly, with mixing, a well-cooled mixture of 1 mL of nitric acid and 1.2 mL of water. Allow to stand for 24 to 48 hours, and pass through a sintered-glass filter. To the filtrate add 0.5 mL of dibasic sodium phosphate TS: a yellow precipitate is formed, and it dissolves in an excess of 6 N ammonium hydroxide.

**Pyrogen** (151)—It meets the requirements, the test dose being 10 mL of Injection per kg.

**pH** (791): between 3.0 and 6.0.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements—**It meets the requirements under *Injections and Implanted Drug Products* (1).

#### Assay—

**Ammonium hydroxide diluent—**Dilute 40 mL of ammonium hydroxide with water to 1000 mL. Store in a plastic bottle.

**Sodium sulfate solution—**Dissolve 1 g of sodium sulfate in water to make 100 mL.

**Molybdenum stock solution—**Transfer about 1.84 g of previously assayed Ammonium Molybdate, accurately weighed, to a 1000-mL volumetric flask, dilute with *Ammonium hydroxide diluent* to volume, and mix. This solution contains the equivalent of 1000 µg of molybdenum per mL.



**Standard preparations**—Transfer 0, 1.0, 2.0, 3.0, and 4.0 mL, respectively, of Molybdenum stock solution to separate 100-mL volumetric flasks, and to the respective flasks add 5.0, 4.0, 3.0, 2.0, and 1.0 mL of Ammonium hydroxide diluent. To each flask add 10 mL of Sodium sulfate solution, dilute with water to volume, and mix. These Standard preparations contain, respectively, 0, 10, 20, 30, and 40 µg of molybdenum per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 500 µg of molybdenum, to a 25-mL volumetric flask, add 1.25 mL of Ammonium hydroxide diluent and 2.5 mL of Sodium sulfate solution, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the Standard preparations and the Assay preparation at the molybdenum emission line of 313.3 nm with a suitable atomic absorption spectrophotometer (see Atomic Absorption Spectroscopy (852)) equipped with a molybdenum hollow-cathode lamp and a nitrous oxide-acetylene reducing flame, using water as the blank. Plot the absorbances of the Standard preparations versus concentration, in µg per mL, of molybdenum, and draw the straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, in µg per mL, of molybdenum in the Assay preparation. Calculate the quantity, in µg, of molybdenum (Mo) in each mL of the Injection taken by the formula:

$$25C/V$$

in which C is the concentration, in µg per mL, of molybdenum in the Assay preparation; and V is the volume, in mL, of Injection taken.

## Amobarbital Sodium

$C_{11}H_{17}N_2NaO_3$  248.25  
2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(3-methylbutyl)-, monosodium salt.  
Sodium 5-ethyl-5-isopentylbarbiturate [64-43-7].

» Amobarbital Sodium contains not less than 98.5 percent and not more than 100.5 percent of  $C_{11}H_{17}N_2NaO_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

### USP Reference standards (11)—

USP Amobarbital RS  
USP Endotoxin RS

**Completeness of solution** (641)—Mix 1.0 g with 10 mL of carbon dioxide-free water; after 1 minute, the solution is clear and free from undissolved solid.

### Identification—

**A: Infrared Absorption** (197K): of residue obtained in the Assay.

**B:** Ignite about 200 mg; the residue effervesces with acid and responds to the tests for Sodium (191).

**pH** (791): not more than 11.0, in the solution prepared for the test for Completeness of solution.

**Loss on drying** (731)—Dry about 1 g, accurately weighed, at 105° for 4 hours; it loses not more than 2.0% of its weight.

### Delete the following:

• **Heavy metals, Method II** (231): 0.003%. • (Official 1-Jan-2018)

**Other requirements**—Where the label states that Amobarbital Sodium is sterile, it meets the requirements for Sterility and Bacterial endotoxins under Amobarbital Sodium for Injection. Where the label states that Amobarbital Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for Bacterial endotoxins under Amobarbital Sodium for Injection.

**Assay**—Dissolve about 500 mg of Amobarbital Sodium, accurately weighed, in about 15 mL of water in a separator. To the solution add 2 mL of hydrochloric acid, shake, and completely extract the liberated amobarbital with 25-mL portions of chloroform. Test for completeness of extraction by extracting with an additional 10-mL portion of chloroform and evaporating the solvent: not more than 0.5 mg of residue remains. Filter the combined extract through a glass filter funnel into a tared beaker, and wash the separator and the filter with several small portions of chloroform. Evaporate the combined filtrate and washings on a steam bath with the aid of a current of air, dry the residue at 105° for 30 minutes, cool, and weigh. The weight of the residue, multiplied by 1.097, represents the weight of  $C_{11}H_{17}N_2NaO_3$ .

## Amobarbital Sodium for Injection

### DEFINITION

Amobarbital Sodium for Injection is Amobarbital Sodium suitable for parenteral use. It contains NLT 98.5% and NMT 100.5% of the labeled amount of amobarbital sodium ( $C_{11}H_{17}N_2NaO_3$ ), calculated on the dried basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Sample:** Residue obtained in the Assay

**Acceptance criteria:** Meets the requirements

#### • B. IDENTIFICATION TESTS—GENERAL, Sodium (191)

**Sample:** Nominally 200 mg of amobarbital sodium from Amobarbital Sodium for Injection

**Analysis:** Ignite the Sample.

**Acceptance criteria:** The residue effervesces with acid and meets the requirements of the tests.

### ASSAY

#### • PROCEDURE

**Sample solution:** Transfer nominally 500 mg of amobarbital sodium from Amobarbital Sodium for Injection to a suitable separator, and dissolve in 15 mL of water. Add 2 mL of hydrochloric acid, and shake. Completely extract the amobarbital with 25-mL portions of chloroform. Combine the chloroform extractions, and pass through a glass filter funnel into a tared beaker. Wash the separator and the filter with several small portions of chloroform. Use the combined chloroform extractions and the washings.

**Test for completeness of extraction:** Extract the remaining solution in the separator with an additional 10-mL portion of chloroform, and evaporate the solvent; NMT 0.5 mg of residue remains.

**Analysis:** Evaporate the Sample solution on a steam bath with the aid of a current of air. Dry the residue at 105° for 30 min, cool, and weigh.

Calculate the percentage of the labeled amount of amobarbital sodium ( $C_{11}H_{17}N_2NaO_3$ ) in the portion of Amobarbital Sodium for Injection taken:

$$\text{Result} = (W_R/W_S) \times (M_{r1}/M_{r2}) \times 100$$



- $W_R$  = weight of the residue from the *Analysis* (mg)  
 $W_S$  = nominal weight of amobarbital sodium in the *Sample solution* (mg)  
 $M_{r1}$  = molecular weight of amobarbital sodium, 248.25  
 $M_{r2}$  = molecular weight of amobarbital, 226.27  
 Acceptance criteria: 98.5%–100.5% on the dried basis

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

**IMPURITIES**

Delete the following:

- **HEAVY METALS, Method II (231):** NMT 30 ppm • (Official 1, Jan-2018)

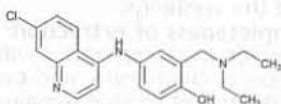
**SPECIFIC TESTS**

- **INJECTIONS AND IMPLANTED DRUG PRODUCTS (1), Specific Tests, Completeness and clarity of solutions:** At the time of use, it meets the requirements.
- **COMPLETENESS OF SOLUTION (641)**  
*Sample solution:* 1 g of amobarbital sodium from Amobarbital Sodium for Injection in 10 mL of carbon dioxide-free water  
*Acceptance criteria:* After 1 min, the solution is clear and free from undissolved solid.
- **LOSS ON DRYING (731)**  
*Sample:* 1 g  
*Analysis:* Dry the *Sample* at 105° for 4 h.  
*Acceptance criteria:* NMT 1.0%
- **pH (791)**  
*Sample solution:* Nominally 100 mg/mL of amobarbital sodium from Amobarbital Sodium for Injection in carbon dioxide-free water  
*Acceptance criteria:* NMT 11.0
- **STERILITY TESTS (71):** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.4 USP Endotoxin Units/mg of amobarbital sodium.
- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling (7)*, *Labels and Labeling for Injectable Products*.

**ADDITIONAL REQUIREMENTS**

Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in • *Packaging and Storage Requirements (659)*, *Injection Packaging, Packaging for constitution* • (CN 1-May-2017).
- **USP REFERENCE STANDARDS (11)**  
 USP Amobarbital RS  
 USP Endotoxin RS

**Amodiaquine**

$C_{20}H_{22}ClN_3O$  355.86  
 Phenol, 4-[(7-chloro-4-quinolyl)amino]-2-[(diethylamino)methyl]-;  
 4-[(7-Chloro-4-quinolyl)amino]- $\alpha$ -(diethylamino)-o-cresol [86-42-0].

**DEFINITION**

Amodiaquine contains NLT 97.0% and NMT 103.0% of amodiaquine ( $C_{20}H_{22}ClN_3O$ ), calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**  
*Standard:* Dissolve 20 mg of USP Amodiaquine Hydrochloride RS in 10 mL of water in a separator, add 1 mL of ammonium hydroxide, and extract by shaking with 25 mL of chloroform. Draw off and evaporate the chloroform extract, and dry the residue at 105° for 2 h.  
*Acceptance criteria:* Meets the requirements
- **B. ULTRAVIOLET ABSORPTION (197U)**  
*Sample solution:* 10  $\mu$ g/mL in 0.1 N hydrochloric acid  
*Acceptance criteria:* Meets the requirements

**ASSAY**

- **PROCEDURE**  
*Standard solution:* 15  $\mu$ g/mL of USP Amodiaquine Hydrochloride RS in 0.1 N hydrochloric acid  
*Sample solution:* 15  $\mu$ g/mL of Amodiaquine in 0.1 N hydrochloric acid  
**Instrumental conditions**  
*(See Ultraviolet-Visible Spectroscopy (857).)*  
**Mode:** UV  
**Analytical wavelength:** 342 nm  
**Cell:** 1 cm  
**Blank:** 0.1 N hydrochloric acid  
**Analysis**  
*Samples:* *Standard solution* and *Sample solution*  
 Calculate the percentage of amodiaquine ( $C_{20}H_{22}ClN_3O$ ) in the portion of Amodiaquine taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of USP Amodiaquine Hydrochloride RS in the *Standard solution* ( $\mu$ g/mL)  
 $C_U$  = concentration of Amodiaquine in the *Sample solution* ( $\mu$ g/mL)  
 $M_{r1}$  = molecular weight of amodiaquine, 355.86  
 $M_{r2}$  = molecular weight of anhydrous aminodiaquine hydrochloride, 428.79  
**Acceptance criteria:** 97.0%–103.0% on the anhydrous basis

**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 0.2%
- **ORGANIC IMPURITIES**  
*Standard solution A:* To 20 mg of USP Amodiaquine Hydrochloride RS in a glass-stoppered test tube add 1.0 mL of chloroform (saturated with ammonium hydroxide), and shake vigorously for 2 min. Allow the solids to settle, and decant the liquid into a second test tube.  
*Standard solution B:* *Standard solution A* and chloroform (saturated with ammonium hydroxide) (1 in 200)  
*Sample solution:* 15 mg/mL of Amodiaquine in chloroform (saturated with ammonium hydroxide)  
**Chromatographic system**  
*(See Chromatography (621), Thin-Layer Chromatography.)*  
**Mode:** TLC  
**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture  
**Application volume:** 10  $\mu$ L  
**Developing solvent system:** Chloroform (saturated with ammonium hydroxide) and dehydrated alcohol (9:1)  
**Analysis**  
*Samples:* *Standard solution A*, *Standard solution B*, and *Sample solution*



Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength UV light.

**Acceptance criteria:** The chromatograms show principal spots at about the same  $R_f$  value; and no secondary spot, if present in the chromatogram from the *Sample solution*, is more intense than the principal spot obtained from *Standard solution B*.

#### SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* (921): NMT 0.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Amodiaquine Hydrochloride RS

### Amodiaquine Hydrochloride

$C_{20}H_{22}ClN_3O \cdot 2HCl \cdot 2H_2O$	464.81
$C_{20}H_{22}ClN_3O \cdot 2HCl$	428.79
Phenol, 4-[(7-chloro-4-quinolinyl)amino]-2-[(diethylamino)-methyl]-, dihydrochloride, dihydrate;	
4-[(7-Chloro-4-quinolyl)amino]- $\alpha$ -(diethylamino)- <i>o</i> -cresol dihydrochloride dihydrate [6398-98-7].	
Anhydrous [69-44-3].	

#### DEFINITION

Amodiaquine Hydrochloride contains NLT 97.0% and NMT 103.0% of amodiaquine hydrochloride ( $C_{20}H_{22}ClN_3O \cdot 2HCl$ ), calculated on the anhydrous basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (17K)

**Sample:** Dissolve 20 mg of Amodiaquine Hydrochloride in 10 mL of water in a separator. Add 1 mL of ammonium hydroxide, and extract by shaking with 25 mL of chloroform. Draw off and evaporate the chloroform extract, and dry the residue at 105° for 2 h.

**Acceptance criteria:** Meets the requirements

- **B. ULTRAVIOLET ABSORPTION** (17U)

**Sample solution:** 10  $\mu$ g/mL in dilute hydrochloric acid (1 in 100)

**Acceptance criteria:** Meets the requirements

- **C. IDENTIFICATION TESTS—GENERAL**, *Chloride* (191): Meets the requirements

- **D.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

- **PROCEDURE**

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Add 1.0 mL of perchloric acid to each 1 L of solution, adjust with phosphoric acid to a pH of 2.5  $\pm$  0.5, and pass through a filter of 0.45- $\mu$ m pore size.

**Mobile phase:** Methanol and *Buffer* (22:78)

**System suitability solution:** 0.15 mg/mL of USP Amodiaquine Hydrochloride RS and 0.15 mg/mL of USP Chloroquine Phosphate RS in water

**Standard solution:** 0.15 mg/mL of USP Amodiaquine Hydrochloride RS in water

**Sample solution:** 0.15 mg/mL in water

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 224 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu$ m packing L1

**Column temperature:** 25°  $\pm$  5°

**Flow rate:** 1.2 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for chloroquine phosphate and amodiaquine hydrochloride are 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between amodiaquine hydrochloride and chloroquine phosphate

**Tailing factor:** NMT 1.5 for amodiaquine hydrochloride and chloroquine phosphate

**Relative standard deviation:** NMT 2.0% for amodiaquine hydrochloride and chloroquine phosphate

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amodiaquine hydrochloride ( $C_{20}H_{22}ClN_3O \cdot 2HCl$ ) in the portion of Amodiaquine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = response from the *Sample solution*

$r_S$  = response from the *Standard solution*

$C_S$  = concentration of USP Amodiaquine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amodiaquine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–103.0% on the anhydrous basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

- **ORGANIC IMPURITIES**

**Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Amodiaquine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = response of each impurity from the *Sample solution*

$r_T$  = sum of the responses from the *Sample solution*

**Acceptance criteria**

**Individual impurity:** NMT 0.5%

#### SPECIFIC TESTS

- **WATER DETERMINATION**, *Method I* (921): 7.0%–9.0%
- **COMPLETENESS OF SOLUTION** (641): A solution of 200 mg in 10 mL of water is clear.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Amodiaquine Hydrochloride RS  
USP Chloroquine Phosphate RS

### Amodiaquine Hydrochloride Tablets

#### DEFINITION

Amodiaquine Hydrochloride Tablets contain an amount of amodiaquine hydrochloride ( $C_{20}H_{22}ClN_3O \cdot 2HCl \cdot 2H_2O$ )



equivalent to NLT 93.0% and NMT 107.0% of the labeled amount of amodiaquine ( $C_{20}H_{22}ClN_3O$ ).

## IDENTIFICATION

### A. INFRARED ABSORPTION (197K)

**Sample:** Powder 1 or more Tablets, and transfer a portion of the powder, equivalent to 50 mg of amodiaquine, to a 125-mL separator. Add 20 mL of water, and shake for 1 min. Add 25 mL of chloroform and 1 mL of ammonium hydroxide, shake for 2 min, and when settled, filter the chloroform extract through cotton that previously has been rinsed with chloroform, collecting the extract in a vessel suitable for evaporation. Evaporate the chloroform, and dry the residue at 105° for 1 h.

**Acceptance criteria:** Meet the requirements

- B.** The retention time of the amodiaquine hydrochloride peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

## ASSAY

### PROCEDURE

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Add 1.0 mL of perchloric acid to each 1 L of solution, adjust with phosphoric acid to a pH of 2.5, and pass through a filter of 0.45-μm pore size.

**Diluent:** 1% (v/v) hydrochloric acid in water

**Mobile phase:** Methanol and *Buffer* (22:78)

**Standard solution:** 0.15 mg/mL of USP Amodiaquine Hydrochloride RS and 0.15 mg/mL of USP Chloroquine Phosphate RS in water

**Sample solution:** Transfer a quantity equivalent to 7.5 mg of amodiaquine hydrochloride from finely powdered Tablets (NLT 20) to a 50-mL volumetric flask, and dissolve in and dilute with *Diluent* to volume. Sonicate for 25 min at 29°. Pass 10 mL through a nylon filter of 0.2-μm pore size, discarding the first 4 mL. Use 2 mL for the analysis.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 224 nm

**Column:** 4.6-mm × 10-cm; 5-μm packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 10 μL

### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for the chloroquine and amodiaquine peaks are 0.8 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 1.5 between amodiaquine hydrochloride and chloroquine phosphate

**Tailing factor:** NMT 1.5 for amodiaquine hydrochloride and chloroquine phosphate

**Relative standard deviation:** NMT 2.0% for amodiaquine hydrochloride and chloroquine phosphate

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of amodiaquine ( $C_{20}H_{22}ClN_3O$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$	= peak response from the <i>Sample solution</i>
$r_S$	= peak response from the <i>Standard solution</i>
$C_S$	= concentration of amodiaquine in USP Amodiaquine Hydrochloride RS in the <i>Standard solution</i> (mg/mL)
$C_U$	= nominal concentration of amodiaquine in the <i>Sample solution</i> (mg/mL)

**Acceptance criteria:** 93.0%–107.0%

## PERFORMANCE TESTS

### DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Detector:** UV 342 nm

**Standard solution:** USP Amodiaquine Hydrochloride RS in *Medium*

**Sample solution:** Filter portions of the solution under test, suitably diluted with water, if necessary, in comparison with a *Standard solution* having a known concentration of USP Amodiaquine Hydrochloride RS.

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Determine the amount of amodiaquine hydrochloride ( $C_{20}H_{22}ClN_3O \cdot 2HCl \cdot 2H_2O$ ) dissolved from UV absorbances.

**Tolerances:** An amount of amodiaquine hydrochloride ( $C_{20}H_{22}ClN_3O \cdot 2HCl \cdot 2H_2O$ ) equivalent to NLT 75% (Q) of the labeled amount of amodiaquine ( $C_{20}H_{22}ClN_3O$ ) is dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## ADDITIONAL REQUIREMENTS

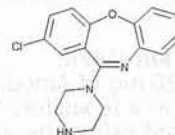
- PACKAGING AND STORAGE:** Preserve in tight containers.

### USP REFERENCE STANDARDS (11)

USP Amodiaquine Hydrochloride RS

USP Chloroquine Phosphate RS

## Amoxapine



$C_{17}H_{16}ClN_3O$  313.78  
Dibenz[b,f][1,4]oxazepine, 2-chloro-11-(1-piperazinyl)-;  
2-Chloro-11-(1-piperazinyl)dibenz[b,f][1,4]oxazepine  
[14028-44-5].

## DEFINITION

Amoxapine contains NLT 98.0% and NMT 102.0% of amoxapine ( $C_{17}H_{16}ClN_3O$ ), calculated on the dried basis.

## IDENTIFICATION

### A. INFRARED ABSORPTION (197K)

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

## ASSAY

### PROCEDURE

**Buffer:** 3.9 g/L of ammonium acetate in water adjusted with acetic acid or diluted ammonia solution to a pH of 7.3

**Mobile phase:** Acetonitrile and *Buffer* (30:70)

**Diluent:** Acetonitrile and *Buffer* (70:30)

**System suitability solution:** 0.1 mg/mL each of USP Amoxapine RS and USP Amoxapine Related Compound G RS in *Diluent*

**Standard solution:** 0.1 mg/mL of USP Amoxapine RS in *Diluent*

**Sample solution:** 0.1 mg/mL of Amoxapine in *Diluent*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 254 nm  
 Column: 4.6-mm × 7.5-cm; 2.5-μm or 2.7-μm packing L1  
 Column temperature: 35°  
 Flow rate: 1.2 mL/min  
 Injection volume: 10 μL

#### System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for amoxapine and amoxapine related compound G are 1.0 and 1.3, respectively.]

#### Suitability requirements

Resolution: NLT 1.5 between amoxapine and amoxapine related compound G, *System suitability solution*

Tailing factor: 0.8–1.8, *Standard solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amoxapine ( $C_{17}H_{16}ClN_3O$ ) in the portion of Amoxapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of amoxapine from the *Sample solution*

$r_S$  = peak response of amoxapine from the *Standard solution*

$C_S$  = concentration of USP Amoxapine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amoxapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

#### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **ORGANIC IMPURITIES**

**Solution A:** 3.9 g/L of ammonium acetate in water adjusted with acetic acid or diluted ammonia solution to a pH of 7.3

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
5	70	30
7.5	60	40
15	60	40
20	20	80
25	20	80
30	70	30
35	70	30

**Diluent:** *Solution A* and *Solution B* (30:70)

**System suitability solution:** 1 mg/mL of USP Amoxapine RS and 1.5 μg/mL of USP Amoxapine Related Compound G RS in *Diluent*

**Standard solution:** 1 μg/mL of USP Amoxapine RS, and 1.5 μg/mL each of USP Amoxapine Related Compound G RS and USP Amoxapine Related Compound D RS in *Diluent*

**Sample solution:** 1000 μg/mL of Amoxapine in *Diluent*

#### Chromatographic system

Proceed as directed in the *Assay*.

#### System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for the relative retention times.]

#### Suitability requirements

Peak-to-valley ratio: NLT 3 between amoxapine and amoxapine related compound G, *System suitability solution*

Relative standard deviation: NMT 5.0% each for amoxapine, amoxapine related compound G, and amoxapine related compound D, *Standard solution*

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of amoxapine related compound G and amoxapine related compound D in the portion of Amoxapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of amoxapine related compound G or amoxapine related compound D from the *Sample solution*

$r_S$  = peak response of amoxapine related compound G or amoxapine related compound D from the *Standard solution*

$C_S$  = concentration of USP Amoxapine Related Compound G RS or USP Amoxapine Related Compound D RS in the *Standard solution* (μg/mL)

$C_U$  = concentration of Amoxapine in the *Sample solution* (μg/mL)

Calculate the percentage of any other impurity in the portion of Amoxapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any other impurity from the *Sample solution*

$r_S$  = peak response of amoxapine from the *Standard solution*

$C_S$  = concentration of USP Amoxapine RS in the *Standard solution* (μg/mL)

$C_U$  = concentration of Amoxapine in the *Sample solution* (μg/mL)

Acceptance criteria: See Table 2. Disregard peaks that are less than 0.02% of the amoxapine peak.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorophenoxy-aniline urea analog <sup>a</sup>	0.57	0.10
Amoxapine	1.0	—
Amoxapine related compound G	1.4	0.15
Amoxapine related compound D	1.7	0.15
Chlorophenoxy-aniline <sup>b</sup>	2.9	0.10
Chlorophenoxy-aniline carbamate <sup>c</sup>	3.8	0.10
N-Carbamoyl amoxapine <sup>d</sup>	4.3	0.10
Amoxapine dimer <sup>e</sup>	5.0	0.10

<sup>a</sup> N-[2-(4-Chlorophenoxy)phenyl]piperazine-1-carboxamide.

<sup>b</sup> 2-(4-Chlorophenoxy)aniline.

<sup>c</sup> Ethyl [2-(4-Chlorophenoxy)phenyl]carbamate.

<sup>d</sup> 4-(2-Chlorodibenzo[b,f][1,4]oxazepin-11-yl)-N-[2-(4-chlorophenoxy)phenyl]piperazine-1-carboxamide.

<sup>e</sup> 1,4-Bis(2-chlorodibenzo[b,f][1,4]oxazepin-11-yl)piperazine.



Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	0.10
Total impurities	—	0.50

<sup>a</sup> N-[2-(4-Chlorophenoxy)phenyl]piperazine-1-carboxamide.

<sup>b</sup> 2-(4-Chlorophenoxy)aniline.

<sup>c</sup> Ethyl [2-(4-Chlorophenoxy)phenyl]carbamate.

<sup>d</sup> 4-(2-Chlorodibenzo[b,f][1,4]oxazepin-11-yl)-N-[2-(4-chlorophenoxy)phenyl]piperazine-1-carboxamide.

<sup>e</sup> 1,4-Bis(2-chlorodibenzo[b,f][1,4]oxazepin-11-yl)piperazine.

## SPECIFIC TESTS

### • LOSS ON DRYING (731)

Analysis: Dry at 105° for 4 h.

Acceptance criteria: NMT 0.5%

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in tight containers.

### • USP REFERENCE STANDARDS (11)

USP Amoxapine RS

USP Amoxapine Related Compound D RS

2-Chlorodibenzo[b,f][1,4]oxazepin-11-one.

C<sub>13</sub>H<sub>8</sub>ClNO<sub>2</sub> 245.66

USP Amoxapine Related Compound G RS

3-Chloro-11-(piperazin-1-yl)dibenzo[b,f][1,4]oxazepine.

C<sub>17</sub>H<sub>16</sub>ClN<sub>3</sub>O 313.78

## Amoxapine Tablets

### DEFINITION

Amoxapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of amoxapine (C<sub>17</sub>H<sub>16</sub>ClN<sub>3</sub>O).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Sample:** Triturate a quantity of finely ground Tablets, equivalent to 50 mg of amoxapine, with 10 mL of chloroform, and filter. Evaporate the filtrate on a steam bath to dryness (about 30 min).

#### • B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Solution A:** 1.38 g/L of monobasic sodium phosphate in water

**Solution B:** 113 g/L of tetramethylammonium chloride in water

**Mobile phase:** Transfer 20.0 mL of *Solution B*, 4.0 mL of dilute phosphoric acid (1 in 5), and 720 mL of acetonitrile to a 2000-mL volumetric flask. Dilute with *Solution A* to volume.

**Standard stock solution:** 1 mg/mL of USP Amoxapine RS in acetonitrile. Shake by mechanical means to dissolve, and then dilute with acetonitrile to volume.

**Standard solution:** 0.1 mg/mL from the *Standard stock solution* diluted with *Mobile phase*

**Sample stock solution:** Nominally 1 mg/mL of amoxapine from NLT 20 finely powdered Tablets prepared as follows. Transfer a suitable quantity of the powder to a volumetric flask. Add 80% of the flask volume of *Mobile phase*, and shake vigorously by mechanical means for 20 min. Dilute with *Mobile phase* to volume, and filter.

**Sample solution:** 0.1 mg/mL from the *Sample stock solution* diluted with *Mobile phase*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

Column efficiency: NLT 1200 theoretical plates

Tailing factor: NMT 1.8

Relative standard deviation: NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amoxapine (C<sub>17</sub>H<sub>16</sub>ClN<sub>3</sub>O) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of amoxapine from the *Sample solution*

$r_S$  = peak response of amoxapine from the *Standard solution*

$C_S$  = concentration of USP Amoxapine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amoxapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

**Medium:** Simulated gastric fluid (without enzyme); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Sample solution:** Sample per *Dissolution* (711).

**Standard solution:** USP Amoxapine RS having a concentration similar to the expected *Sample solution* in *Medium*

### Instrumental conditions

Analytical wavelength: 294 nm

### Analysis

**Samples:** *Sample solution* and *Standard solution*

Determine the percentage of the labeled amount of amoxapine (C<sub>17</sub>H<sub>16</sub>ClN<sub>3</sub>O) dissolved from UV absorbances of filtered portions of the *Sample solution*, suitably diluted with *Medium*, if necessary.

Calculate the percentage of the labeled amount of amoxapine (C<sub>17</sub>H<sub>16</sub>ClN<sub>3</sub>O) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of amoxapine (C<sub>17</sub>H<sub>16</sub>ClN<sub>3</sub>O) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

## ADDITIONAL REQUIREMENTS

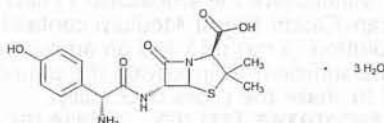
### • PACKAGING AND STORAGE: Preserve in well-closed containers.

### • USP REFERENCE STANDARDS (11)

USP Amoxapine RS



## Amoxicillin



$C_{16}H_{19}N_3O_5S \cdot 3H_2O$  419.45  
 4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-, trihydrate [2S-[2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ (S\*)]]-; (2S,5R,6R)-6-[(R)-(-)-2-Amino-2-(p-hydroxyphenyl)-acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate [61336-70-7].  
 Anhydrous 365.41  
 [26787-78-0].

### DEFINITION

Amoxicillin contains NLT 900  $\mu$ g and NMT 1050  $\mu$ g of  $C_{16}H_{19}N_3O_5S$  per mg, calculated on the anhydrous basis.

### IDENTIFICATION

- **INFRARED ABSORPTION** (197K)

### ASSAY

- **PROCEDURE**

**Diluent:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of  $5.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Diluent* (1:24)

**Standard solution:** 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. [NOTE—Use this solution within 6 h.]

**Sample solution:** 1.2 mg/mL of Amoxicillin in *Diluent*. [NOTE—Use this solution within 6 h.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in  $\mu$ g/mg, of  $C_{16}H_{19}N_3O_5S$  in the portion of Amoxicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of *Sample solution* (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS ( $\mu$ g/mg)

**Acceptance criteria:** 900–1050  $\mu$ g of  $C_{16}H_{19}N_3O_5S$  per mg on the anhydrous basis

### IMPURITIES

#### Organic Impurities

- **PROCEDURE**

**Solution A:** 2.72 g/L of monobasic potassium phosphate. Adjust with 1 N potassium hydroxide or 20% phosphoric acid to a pH of  $5.0 \pm 0.1$ .

**Solution B:** Methanol

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	97	3
10	97	3
22	75	25
26	97	3

**Standard solution:** 12.5  $\mu$ g/mL of USP Amoxicillin RS in *Solution A*

**System suitability solution:** 12.5  $\mu$ g/mL each of USP Amoxicillin Related Compound A RS and USP Amoxicillin Related Compound D RS in *Solution A*

**Sample solution:** 1.25 mg/mL of Amoxicillin in *Solution A*. [NOTE—Store this solution at 4° and use within 4 h.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

**Autosampler temperature:** 4°

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

[NOTE—Identify peaks by the relative retention times in *Impurity Table 1*.]

**Resolution:** NLT 1.5 between amoxicillin related compound A and the second peak for amoxicillin related compound D, *System suitability solution*

**Relative standard deviation:** NMT 10%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Amoxicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of amoxicillin from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of Amoxicillin in the *Sample solution* (mg/mL)

$F$  = unit conversion factor (0.001 mg/ $\mu$ g)

#### Acceptance criteria

[NOTE—The reporting limit is 0.03 times the amoxicillin peak from the *Standard solution*.]

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 5.0%



Impurity Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amoxicillin related compound Ia (D-hydroxyphenylglycine)	0.32	1.0
Amoxicillin related compound Db,c (amoxicillin open ring)	0.53	1.0
	0.68	1.0
Amoxicillin related compound Ad (6-aminopenicillanic acid)	0.78	0.5
Amoxicillin related compound Be,f (L-amoxicillin)	0.87	—
Amoxicillin	1.0	—
Amoxicillin related compound Gg (D-hydroxyphenylglycylamoxicillin)	2.9	1.0
Amoxicillin related compound Eh,i (amoxicillin penilloic derivative)	4.5	1.0
Amoxicillin related compound Mi (N-(penicillan-6-yl) open ring amoxicillinamide)	6.0	1.0
Amoxicillin related compound Fe,k (phenylpyrazinediol)	6.3	—
Amoxicillin related compound Cl (amoxicillin rearrangement product)	6.4	1.0
Amoxicillin related compound Eh,i (amoxicillin penilloic derivative)	6.7	1.0
Amoxicillin related compound Jm (amoxicillin open ring dimer)	8.8	1.0
Amoxicillin related compound Ln (N-(penicillan-6-yl) amoxicillinamide)	9.0	1.0
Any unspecified individual impurity	—	1.0

<sup>a</sup> (R)-2-Amino-2-(4-hydroxyphenyl)acetic acid.

<sup>b</sup> The chromatographic system resolves two penicilloic acids from each other.

<sup>c</sup> (4S)-2-[[[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>d</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid.

<sup>e</sup> These compounds are listed for information only and are not to be reported.

<sup>f</sup> (2S,5R,6R)-6-[(S)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>g</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid.

<sup>h</sup> The chromatographic system resolves two penilloic acids from each other.

<sup>i</sup> (4S)-2-[[[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>j</sup> (2S,5R,6R)-6-[(2R)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidine-2-yl]acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>k</sup> 3-(4-Hydroxyphenyl)pyrazin-2-ol.

<sup>l</sup> (4S)-2-[5-(4-Hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>m</sup> (2S,5R,6R)-6-[(2R)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidine-2-yl]acetamido]-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid.

<sup>n</sup> (2S,5R,6R)-6-[(2S,5R,6R)-6-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

## SPECIFIC TESTS

- **CRYSTALLINITY** (695): Meets the requirements
- **DIMETHYLANILINE** (223): Meets the requirement
- **pH** (791): 3.5–6.0

Sample solution: 2 mg/mL

- **WATER DETERMINATION, Method I** (921): 11.5%–14.5%
- **STERILITY TESTS** (71): Where the label states that Amoxicillin is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*,

*Direct Inoculation of the Culture Medium*, except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (5 mg/mL) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, to use Soybean–Casein Digest Medium containing polysorbate 80 solution (5 mg/mL) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, and to shake the tubes once daily.

- **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that Amoxicillin is sterile or Amoxicillin must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.25 USP Endotoxin Unit/mg of amoxicillin.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is intended for veterinary use only and that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Label all other Amoxicillin to indicate that it is to be used in the manufacture of nonparenteral drugs only.

## USP REFERENCE STANDARDS (11)

USP Amoxicillin RS

USP Amoxicillin Related Compound A RS

(2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid; 6-aminopenicillanic acid.

C<sub>8</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>S 216.26

USP Amoxicillin Related Compound D RS

(4S)-2-[[[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid; amoxicillin open ring.

C<sub>16</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>S 383.42

USP Endotoxin RS

## Amoxicillin Boluses

» Amoxicillin Boluses contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of amoxicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>S).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Labeling**—Label Boluses to indicate that they are for veterinary use only.

## USP Reference standards (11)—

USP Amoxicillin RS

## Identification—

**Test solution**—To a portion of powdered Boluses add 0.1 N hydrochloric acid to obtain a *Test solution* containing about 4 mg of amoxicillin per mL. Use within 10 minutes after preparation.

**Application volume, Developing solvent system, Procedure**—Proceed as directed for the *Identification* test under *Amoxicillin Tablets*.

**Disintegration** (701): 30 minutes, simulated gastric fluid being used instead of water.

**Water Determination, Method I** (921): not more than 7.5%.

## Assay—

**Diluent, Mobile phase, Standard preparation, and Chromatographic system**—Prepare as directed in the *Assay* under *Amoxicillin*.

**Assay preparation**—Weigh and finely powder not fewer than 5 Boluses. Transfer an accurately weighed portion of



the powder, equivalent to about 250 mg of amoxicillin, to a 250-mL volumetric flask, add *Diluent* to volume, and mix. Sonicate if necessary to ensure complete dissolution of the amoxicillin. Pass a portion of this solution through a filter of 1- $\mu$ m or finer porosity, and use the filtrate as the *Assay preparation*. [NOTE—Use this solution within 6 hours.]

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Amoxicillin*. Calculate the quantity, in mg, of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) in the portion of Boluses taken by the formula:

$$0.25CP(r_u/r_s)$$

in which the terms are as defined therein.

## Amoxicillin Capsules

### DEFINITION

Amoxicillin Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ).

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** Dissolve 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of  $5.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (1:24)

**Standard solution:** 1.2 mg/mL of USP Amoxicillin RS in *Buffer*. [NOTE—Use this solution within 6 h.]

**Sample solution:** Remove, as completely as possible, the contents of NLT 20 Capsules. Mix the combined contents, and transfer a quantity, equivalent to 200 mg of anhydrous amoxicillin, to a 200-mL volumetric flask. Add *Buffer* to volume. Sonicate if necessary to ensure complete dissolution. [NOTE—Use this solution within 6 h.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4-mm  $\times$  25-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{16}H_{19}N_3O_5S$  in the portion of Capsules taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

Acceptance criteria: 90.0%–120.0%

### PERFORMANCE TESTS

#### DISSOLUTION (711)

##### Test 1

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm, for Capsules containing 250 mg

**Apparatus 2:** 75 rpm, for Capsules containing 500 mg

**Time:** 60 min

**Analytical wavelength:** UV 272 nm

**Standard solution:** USP Amoxicillin RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 90 min

**Analytical wavelength:** UV 272 nm

**Standard solution:** USP Amoxicillin RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  is dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

### SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed  $10^3$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g.

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- USP REFERENCE STANDARDS (11)**  
USP Amoxicillin RS

## Amoxicillin Intramammary Infusion

» Amoxicillin Intramammary Infusion is a suspension of Amoxicillin in a suitable vegetable oil vehicle. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ). It contains a suitable dispersing agent and preservative.

**Packaging and storage**—Preserve in well-closed disposable syringes.

**Labeling**—Label it to indicate that it is intended for veterinary use only.

**USP Reference standards (11)**—  
USP Amoxicillin RS

**Identification**—Transfer a quantity of Intramammary Infusion, equivalent to about 60 mg of amoxicillin, to a 50-mL centrifuge tube, add 25 mL of toluene, mix, and centrifuge. Decant and discard the toluene. Wash the residue with four 25-mL portions of toluene, sonicating for about 30 seconds



after each addition of toluene. Dry the residue in vacuum over silica gel. Add 15 mL of 0.1 N hydrochloric acid to the residue, and mix. The solution obtained responds to the *Identification* test under *Amoxicillin Capsules*.

**Water Determination, Method I (921):** not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

#### Change to read:

**Assay**—Proceed as directed for amoxicillin under *Antibiotics—Microbial Assays* (81). Expel the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of *Buffer B.3* (CN 1-May-2017) and 1.0 mL of polysorbate 80, and blend for 3 to 5 minutes. Allow to stand for about 10 minutes, and dilute an accurately measured volume of the aqueous phase quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

### Amoxicillin for Injectable Suspension

» Amoxicillin for Injectable Suspension is a sterile mixture of Amoxicillin and one or more suitable buffers, preservatives, stabilizers, and suspending agents. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ).

#### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

**Labeling**—Label it to indicate that it is for veterinary use only.

#### USP Reference standards (11)—

USP Amoxicillin RS  
USP Endotoxin RS

**Identification**—Prepare a test solution containing the equivalent of 4 mg of amoxicillin per mL by adding 0.1 N hydrochloric acid to Amoxicillin for Injectable Suspension. Allow the solution to stand for 5 minutes before use: the solution responds to the *Identification* test under *Amoxicillin Capsules*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.25 Endotoxin Unit per mg of amoxicillin.

**Sterility Tests** (71)—It meets the requirements when tested as directed in the section *Direct Inoculation of the Culture Medium under Test for Sterility of the Product to be Examined*, except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, to use Soybean-Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, and to shake the tubes once daily.

**pH** (791): between 5.0 and 7.0, in the suspension constituted as directed in the labeling.

**Water Determination, Method I (921):** between 11.0% and 14.0%.

#### Assay—

*Diluent, Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Amoxicillin*.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Amoxicillin for Injectable Suspension as directed in the labeling. Withdraw all of the withdrawable contents, using a hypodermic needle and syringe, and quantitatively dilute with *Diluent* to obtain a solution containing about 1 mg of anhydrous amoxicillin per mL. Pass a portion of this solution through a suitable filter of 1- $\mu$ m or finer porosity, and use the filtrate as *Assay preparation 1*. Use this solution within 6 hours.

*Assay preparation 2* (where the label states the quantity of amoxicillin in a given volume of constituted suspension)—Constitute Amoxicillin for Injectable Suspension as directed in the labeling. Quantitatively dilute an accurately measured volume of the constituted suspension with *Diluent* to obtain a solution containing about 1 mg of anhydrous amoxicillin per mL. Pass a portion of this solution through a suitable filter of 1- $\mu$ m or finer porosity, and use the filtrate as *Assay preparation 2*. Use this solution within 6 hours.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Amoxicillin*. Calculate the quantity, in mg, of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) in the container, or in the portion of constituted Suspension taken by the formula:

$$(L/D)(CP/1000)(r_u/r_s)$$

in which *L* is the labeled quantity, in mg, of anhydrous amoxicillin in the container, or in the volume of constituted suspension taken; *D* is the concentration, in mg of anhydrous amoxicillin per mL, of *Assay preparation 1* or of *Assay preparation 2* on the basis of the labeled quantity in the container or in the portion of constituted suspension taken, respectively, and the extent of dilution; and the other terms are as defined therein.

### Amoxicillin Oral Suspension

» Amoxicillin Oral Suspension is a suspension of Amoxicillin in Soybean Oil. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ).

**Packaging and storage**—Preserve in multiple-dose containers equipped with a suitable dosing pump.

**Labeling**—Label it to indicate that it is for veterinary use only.

#### USP Reference standards (11)—

USP Amoxicillin RS

**Identification**—Shake a portion of Oral Suspension with a mixture of acetone and 0.1 N hydrochloric acid (4:1) to obtain a solution containing about 1 mg of amoxicillin per mL. The solution responds to the *Identification* test under *Amoxicillin Capsules*.

**Water Determination, Method I (921):** not more than 2.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

#### Assay—

*Standard preparation*—Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics* (425), using USP Amoxicillin RS.

*Assay preparation*—Using the dosing pump, deliver a number of doses of Oral Suspension, equivalent to about 250 mg of amoxicillin, to a separator containing 100 mL of hexanes, and shake vigorously. Add 140 mL of water, and



shake for 5 minutes. Allow the layers to separate, and drain the lower, aqueous layer into a 250-mL volumetric flask. Repeat the extraction with two 50-mL portions of water. Combine the aqueous extracts in the volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed with Oral Suspension as directed for *Procedure under Iodometric Assay—Antibiotics* (425), using USP Amoxicillin RS. Calculate the quantity, in mg, of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) in each dose of Oral Suspension taken by the formula:

$$(250 / N)(F / 2000)(B - I)$$

in which *N* is the number of doses taken, and the other terms are as defined therein.

## Amoxicillin for Oral Suspension

### DEFINITION

Amoxicillin for Oral Suspension contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ). It contains one or more suitable buffers, colors, flavors, preservatives, stabilizers, sweeteners, and suspending agents.

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** Dissolve 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of  $5.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (1:24)

**Standard solution:** 1.2 mg/mL of USP Amoxicillin RS in *Buffer*. [NOTE—Use this solution within 6 h.]

**Sample solution:** Dilute a measured volume of Amoxicillin for Oral Suspension, constituted as directed in the labeling, freshly mixed and free from air bubbles, quantitatively and stepwise in *Buffer* to obtain a solution containing nominally 1 mg/mL of anhydrous amoxicillin. Pass a portion of this solution through a suitable filter. [NOTE—Use this solution within 6 h.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4-mm  $\times$  25-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{16}H_{19}N_3O_5S$  in the Amoxicillin for Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of anhydrous amoxicillin in the *Sample solution* (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–120.0%

### PERFORMANCE TESTS

#### UNIFORMITY OF DOSAGE UNITS (905)

For solids packaged in single-unit containers: Meets the requirements

#### DELIVERABLE VOLUME (698):

Meets the requirements

### SPECIFIC TESTS

- pH (791):** 5.0–7.5, in the suspension constituted as directed in the labeling

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed  $10^3$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g.

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**  
USP Amoxicillin RS

## Amoxicillin Tablets

### DEFINITION

Amoxicillin Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ).

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of  $5.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (1:24)

**Standard solution:** 1.2 mg/mL of USP Amoxicillin RS in *Buffer*. [NOTE—Use this solution within 6 h.]

**Sample solution:** Place NLT 5 Tablets in a high-speed glass blender jar containing *Buffer* sufficient to yield a concentration of 1 mg/mL of anhydrous amoxicillin. Blend for  $4 \pm 1$  min, allow to stand for 5 min, and centrifuge a portion of the mixture. [NOTE—Where the volume of *Buffer* required would exceed 500 mL, place 5 Tablets in a volumetric flask of such capacity that when finally diluted to volume, a concentration of 1 mg of anhydrous amoxicillin per mL would be obtained. Add a volume of *Buffer* equivalent to three-fourths of the capacity of the volumetric flask, and sonicate for 5 min. Dilute with *Buffer* to volume, add a magnetic stirring bar, and stir for 30 min. Centrifuge a portion of this solution.]

Pass a portion of the clear supernatant through a suitable filter. [NOTE—Use this solution within 6 h.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 230 nm  
 Column: 4-mm × 25-cm; 10-μm packing L1  
 Flow rate: 1.5 mL/min  
 Injection size: 10 μL  
 System suitability  
 Sample: Standard solution  
 Suitability requirements  
 Tailing factor: NMT 2.5  
 Relative standard deviation: NMT 2.0%

**Analysis**

Samples: Standard solution and Sample solution  
 Calculate the percentage of  $C_{16}H_{19}N_3O_5S$  in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of amoxicillin from the Sample solution  
 $r_S$  = peak response of amoxicillin from the Standard solution  
 $C_S$  = concentration of USP Amoxicillin RS in the Standard solution (mg/mL)  
 $C_U$  = nominal concentration of amoxicillin in the Sample solution (mg/mL)  
 $P$  = potency of amoxicillin in USP Amoxicillin RS (μg/mg)  
 $F$  = conversion factor, 0.001 mg/μg  
 Acceptance criteria: 90.0%–120.0%

**PERFORMANCE TESTS**• **DISSOLUTION** (711)

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Determine the amount of  $C_{16}H_{19}N_3O_5S$  dissolved by using the following method.

Buffer: 27.2 g of monobasic potassium phosphate in 3 L of water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of  $5.0 \pm 0.1$ . Dilute with water to obtain 4 L of solution.

Mobile phase: Acetonitrile and Buffer (1:39)

Standard solution: 0.05 mg/mL of USP Amoxicillin RS in Buffer. [NOTE—Use this solution within 6 h.]

Sample solution: Pass a portion of the sample through a suitable filter of 0.5-μm pore size. Quantitatively dilute a volume of the filtrate with water to obtain an estimated concentration of 0.045 mg/mL of amoxicillin. Use this solution within 6 h.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 230 nm

Column

Analytical: 3.9-mm × 30-cm; packing L1

Guard: 2-mm × 2-cm; packing L2

Column temperature: The analytical column is maintained at a constant temperature of  $40 \pm 1^\circ$ .

Flow rate: 0.7 mL/min

Injection size: 10 μL

System suitability

Sample: Standard solution

Suitability requirements

Capacity factor: 1.1–2.8

Column efficiency: NLT 1700 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 1.5%

**Analysis**

Samples: Standard solution and Sample solution  
 Calculate the percentage of  $C_{16}H_{19}N_3O_5S$  dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times P \times F \times 100$$

$r_U$  = peak response of amoxicillin from the Sample solution

$r_S$  = peak response of amoxicillin from the Standard solution

$C_S$  = concentration of USP Amoxicillin RS in the Standard solution (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of the dissolution medium, 900 mL

$D$  = dilution factor for the Sample solution

$P$  = potency of amoxicillin in USP Amoxicillin RS (μg/mg)

$F$  = conversion factor, 0.001 mg/μg

Tolerances: NLT 75% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  is dissolved.

For products labeled as chewable Tablets: Proceed as directed above.

For chewable Tablets labeled to contain 200 or 400 mg

Time: 20 min

Tolerances: NLT 70% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  is dissolved.

For chewable Tablets labeled to contain 125 or 250 mg

Time: 90 min

Tolerances: NLT 70% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  is dissolved.

For veterinary products: Proceed as directed above, except use Apparatus 2 at 100 rpm.

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed  $10^3$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label chewable Tablets to indicate that they are to be chewed before swallowing. Tablets intended solely for veterinary use are so labeled.
- **USP REFERENCE STANDARDS** (11)  
 USP Amoxicillin RS

**Amoxicillin Tablets for Oral Suspension****DEFINITION**

Amoxicillin Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ).

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

Standard solution: 4 mg/mL of USP Amoxicillin RS in 0.1 N hydrochloric acid. Use within 10 min of preparation.

Sample solution: An aqueous dispersion of Tablets for Oral Suspension in 0.1 N hydrochloric acid containing 4 mg/mL of amoxicillin. Use within 10 min of preparation.

**Chromatographic system**

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5 μL

Developing solvent system: Methanol, chloroform, pyridine, and water (90:80:1:30)

Spray reagent: 3 mg/mL of ninhydrin in alcohol

**Analysis**

Samples: Standard solution and Sample solution

Proceed as directed in the chapter. Dry the plate with the aid of a current of warm air for 10 min. Spray



lightly with *Spray reagent*, and dry at 110° for 15 min.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

## ASSAY

### PROCEDURE

**Diluent:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1.

**Mobile phase:** Acetonitrile and *Diluent* (1:24). Decrease the acetonitrile concentration to increase the retention time of amoxicillin.

**Standard solution:** 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. Use this solution within 6 h.

**Sample solution:** Prepare a dispersion of 20 Tablets for Oral Suspension using a suitable aliquot of water. Dilute a portion of the dispersion with *Diluent* to obtain a solution containing 1.2 mg/mL of amoxicillin. Pass a portion of the solution through a filter of 1-μm or finer pore size. Use this solution within 6 h.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4-mm × 25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 μL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Capacity factor:** 1.1–2.8

**Column efficiency:** NLT 1700 theoretical plates

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) in the portion of Tablets for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS (μg/mg)

$F$  = conversion factor, 0.001 mg/μg

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

### DISINTEGRATION (701)

**Medium:** Water at 20 ± 5°

**Time:** 3 min

**Acceptance criteria:** Meet the requirements

### DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Buffer:** 27.2 g of monobasic potassium phosphate in 3 L of water. Adjust with a 45% (w/w) solution of potassium hydroxide to a pH of 5.0 ± 0.1, and dilute with water to obtain 4 L of solution.

**Mobile phase:** Acetonitrile and *Buffer* (10:390). Pass through a filter of 0.5-μm or finer pore size.

**Standard solution:** 0.05 mg/mL of USP Amoxicillin RS in *Buffer*. Use this solution within 6 h.

**Sample solution:** Pass a portion of the sample through a filter of 0.5-μm or finer pore size. Dilute a suitable

aliquot of the filtrate with water to obtain a concentration of 0.045 mg/mL of amoxicillin. Use this solution within 6 h.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

### Columns

**Guard:** 2-mm × 2-cm; packing L2

**Analytical:** 3.9-mm × 30-cm; packing L1

**Column temperature:** 40 ± 1°

**Flow rate:** 0.7 mL/min

**Injection volume:** 10 μL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Capacity factor:** 1.1–2.8

**Column efficiency:** NLT 1700 theoretical plates

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 1.5%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times D \times P \times F \times (1/L) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$V$  = volume of medium, 900 mL

$D$  = dilution factor

$P$  = potency of amoxicillin in USP Amoxicillin RS (μg/mg)

$F$  = conversion factor, 0.001 mg/μg

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## SPECIFIC TESTS

- **DISPERSION FINENESS:** Place 2 Tablets for Oral Suspension in 100 mL of water, and stir until completely dispersed. A smooth dispersion that passes through a No. 25 sieve is obtained.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
USP Amoxicillin RS

## Amoxicillin and Clavulanate Potassium for Oral Suspension

### DEFINITION

Amoxicillin and Clavulanate Potassium for Oral Suspension contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) and the equivalent of NLT 90.0% and NMT 125.0% of the labeled amount of clavulanic acid ( $C_8H_9NO_5$ ). It contains one or more suitable buffers, colors, flavors, preservatives, stabilizers, sweeteners, and suspending agents.

### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.



**ASSAY****• PROCEDURE**

**Buffer:** 7.8 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of  $4.4 \pm 0.1$ , and dilute with water to 1000 mL.

**Mobile phase:** Methanol and Buffer (1:19). Pass through a suitable filter.

**Standard solution:** 0.5 mg/mL of USP Amoxicillin RS and 0.2 mg/mL of USP Clavulanate Lithium RS in water

**Sample solution:** Nominally 0.5 mg/mL of amoxicillin in water, prepared as follows. Constitute Amoxicillin and Clavulanate Potassium for Oral Suspension with water using the volume specified in the labeling. Stir by mechanical means for 10 min, and filter. Use within 1 h.

**Chromatographic system**

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4-mm  $\times$  30-cm; 3- to 10- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** Standard solution

[NOTE—The relative retention times for clavulanic acid and amoxicillin are about 0.5 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.5 between the amoxicillin and clavulanic acid peaks

**Tailing factor:** NMT 1.5 for each analyte peak

**Relative standard deviation:** NMT 2.0% for each analyte peak

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) in the Amoxicillin and Clavulanate Potassium for Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response of amoxicillin from the Sample solution

$r_s$  = peak response of amoxicillin from the Standard solution

$C_s$  = concentration of USP Amoxicillin RS in the Standard solution (mg/mL)

$C_u$  = nominal concentration of amoxicillin in the Sample solution (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

Calculate the percentage of the labeled amount of clavulanic acid ( $C_8H_9NO_5$ ) in the Amoxicillin and Clavulanate Potassium for Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times 100$$

$r_u$  = peak response of clavulanic acid from the Sample solution

$r_s$  = peak response of clavulanic acid from the Standard solution

$C_s$  = concentration of USP Clavulanate Lithium RS in the Standard solution (mg/mL)

$C_u$  = nominal concentration of clavulanic acid in the Sample solution (mg/mL)

$P$  = potency of clavulanic acid in USP Clavulanate Lithium RS (mg/mg)

**Acceptance criteria:** 90.0%–120.0% of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) and 90.0%–125.0% of the labeled amount of clavulanic acid ( $C_8H_9NO_5$ )

**PERFORMANCE TESTS****• DELIVERABLE VOLUME (698)**

For powder packaged in multiple-unit containers: Meets the requirements

**• UNIFORMITY OF DOSAGE UNITS (905)**

For powder packaged in single-unit containers: Meets the requirements

**SPECIFIC TESTS****• PH (791)**

**Sample solution:** Constitute as directed in the labeling, and perform the test immediately after constitution.

**Acceptance criteria:** 3.8–6.6

**• MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed  $10^3$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g.**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight containers, at controlled room temperature.**• USP REFERENCE STANDARDS (11)**

USP Amoxicillin RS

USP Clavulanate Lithium RS

**Amoxicillin and Clavulanate Potassium Tablets****DEFINITION**

Amoxicillin and Clavulanate Potassium Tablets contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amounts of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) and clavulanic acid ( $C_8H_9NO_5$ ).

**IDENTIFICATION**

- The retention times of the major peaks of the Sample solution correspond to those of the Standard solution, as obtained in the Assay.

**ASSAY****• PROCEDURE**

**Buffer:** 7.8 g of monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of  $4.4 \pm 0.1$ , and dilute with water to 1000 mL.

**Mobile phase:** Methanol and Buffer (1:19). Pass through a suitable filter.

**Standard solution:** 0.5 mg/mL of USP Amoxicillin RS and 0.2 mg/mL of USP Clavulanate Lithium RS in water

**Sample stock solution:** Dissolve NLT 10 Tablets in water with the aid of mechanical stirring. Transfer to a suitable volumetric flask, and dilute with water to volume.

**Sample solution:** Dilute a suitable volume of the Sample stock solution with water to obtain a solution containing 0.5 mg/mL of amoxicillin. [NOTE—Use the Sample solution within 1 h.]

**Chromatographic system**

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4-mm  $\times$  30-cm; 3- to 10- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20  $\mu$ L

**System suitability**

**Sample:** Standard solution

[NOTE—The relative retention times for clavulanic acid and amoxicillin are 0.5 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.5 between the amoxicillin and clavulanic acid peaks



Tailing factor: NMT 1.5 for each analyte peak

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of  $C_{16}H_{19}N_3O_5S$  in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of amoxicillin from the Sample solution

$r_S$  = peak response of amoxicillin from the Standard solution

$C_S$  = concentration of USP Amoxicillin RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of amoxicillin in the Sample solution (mg/mL)

$P$  = potency of USP Amoxicillin RS ( $\mu\text{g}/\text{mg}$ )

$F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

Calculate the percentage of  $C_8H_9NO_5$  in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak response of clavulanic acid from the Sample solution

$r_S$  = peak response of clavulanic acid from the Standard solution

$C_S$  = concentration of USP Clavulanate Lithium RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of clavulanic acid in the Sample solution (mg/mL)

$P$  = potency of clavulanic acid in USP Clavulanate Lithium RS (mg/mg)

Acceptance criteria: 90.0%–120.0%

#### PERFORMANCE TESTS

- **DISINTEGRATION** (701): Tablets labeled for veterinary use only; 30 min, simulated gastric fluid TS being substituted for water in the test

- **DISSOLUTION** (711)

[NOTE—Tablets labeled for veterinary use only are exempt from this requirement.]

##### Test 1

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 30 min; or 45 min where the Tablets are labeled as chewable

Analysis: Determine the amount of  $C_{16}H_{19}N_3O_5S$  and  $C_8H_9NO_5$  dissolved, using the Analysis set forth in the Assay, making any necessary volumetric adjustments.

Tolerances: NLT 85% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  and NLT 80% (Q) of the labeled amount of  $C_8H_9NO_5$  are dissolved.

For Tablets labeled as chewable: NLT 80% (Q) of the labeled amounts of  $C_{16}H_{19}N_3O_5S$  and  $C_8H_9NO_5$  is dissolved in 45 min.

Test 2: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

Medium, Apparatus 2, and Analysis: Proceed as directed for Test 1.

Times: 45 min for amoxicillin, and 30 min for clavulanic acid

Tolerances: NLT 85% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_5S$  and NLT 80% (Q) of the labeled amount of  $C_8H_9NO_5$  are dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### SPECIFIC TESTS

- **WATER DETERMINATION**, Method I (921):

Tablet Label Claim Amoxicillin (mg/Tablet)	Acceptance Criteria, NMT (%)
≤250	7.5
>250 and ≤500	10.0
>500	11.0

For products labeled as chewable Tablets:

Tablet Label Claim Amoxicillin (mg/Tablet)	Acceptance Criteria, NMT (%)
≤125	6.0
>125	8.0

For Tablets labeled for veterinary use only: NMT 10.0%

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed  $10^3$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label chewable Tablets to include the word "chewable" in juxtaposition to the official name. The labeling indicates that chewable Tablets may be chewed before being swallowed or may be swallowed whole. Tablets intended for veterinary use only are so labeled. When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.
- **USP REFERENCE STANDARDS** (11)  
USP Amoxicillin RS  
USP Clavulanate Lithium RS

### Amoxicillin and Clavulanic Acid Extended-Release Tablets

#### DEFINITION

Amoxicillin and Clavulanic Acid Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) and clavulanic acid ( $C_8H_9NO_5$ ).

#### IDENTIFICATION

- **A.** The retention times of the major peaks of the Sample solution correspond to those of the Standard solution, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

Buffer: 6.9 g/L of monobasic sodium phosphate adjusted with phosphoric acid to a pH of 4.2

Mobile phase: Methanol and Buffer (5:95)

Standard solution: 1 mg/mL of USP Amoxicillin RS and 62.5  $\mu\text{g}/\text{mL}$  of USP Clavulanate Lithium RS in water. Store the solution at 4°, and inject within 10 h.

Sample solution: Equivalent to 1 mg/mL of amoxicillin and 62.5  $\mu\text{g}/\text{mL}$  of clavulanic acid from finely powdered Tablets (NLT 6) in water. Stir for about 60 min. Store the solution at 4°, and inject within 12 h.

##### Chromatographic system

(See Chromatography (621), System Suitability.)



Mode: LC  
 Detector: UV 229 nm  
 Column: 8-mm × 10-cm; 5-μm packing L1  
 Flow rate: 2 mL/min  
 Injection volume: 20 μL  
 Autosampler temperature: 4°

**System suitability**

Sample: Standard solution

**Suitability requirements**

Resolution: NLT 2.0 between the amoxicillin and clavulanic acid peaks

Tailing factor: NMT 1.8 for the amoxicillin and clavulanic acid peaks

Relative standard deviation: NMT 1.0% for the amoxicillin and clavulanic acid peaks

**Analysis**

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = response of amoxicillin from the Sample solution

$r_S$  = response of amoxicillin from the Standard solution

$C_S$  = concentration of USP Amoxicillin RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of amoxicillin in the Sample solution (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS (μg/mg)

$F$  = conversion factor, 0.001 mg/μg

Calculate the percentage of the labeled amount of clavulanic acid ( $C_8H_9NO_5$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = response of clavulanic acid from the Sample solution

$r_S$  = response of clavulanic acid from the Standard solution

$C_S$  = concentration of USP Clavulanate Lithium RS in the Standard solution (μg/mL)

$C_U$  = nominal concentration of clavulanic acid in the Sample solution (μg/mL)

$P$  = potency of clavulanic acid in USP Clavulanate Lithium RS (mg/mg)

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION (711)****Test 1**

Medium: Water; 900 mL

Apparatus 2: 75 rpm

**Times**

Amoxicillin: 1, 3, and 5 h

Clavulanic acid: 1 h

Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Standard solution: USP Amoxicillin RS and USP Clavulanate Lithium RS in Medium at known concentrations similar to those expected in the Sample solution

Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with Medium, if necessary.

**Analysis**

Samples: Standard solution and Sample solution

Calculate the amounts of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) and clavulanic acid ( $C_8H_9NO_5$ ) dissolved.

**Tolerances**

Amoxicillin: The percentage of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) dissolved at the times specified conforms to Table 1.

**Table 1**

Time (h)	Amount Dissolved (%)
1	50–65
3	65–85
5	NLT 85

Clavulanic acid: NLT 80% (Q) of the labeled amount of clavulanic acid ( $C_8H_9NO_5$ ) is dissolved in 1 h.

**Test 2**

Medium: Water; 900 mL

Apparatus 2: 75 rpm

**Times**

Amoxicillin: 1, 3, and 5 h

Clavulanic acid: 45 min

Mobile phase, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Standard solution: USP Amoxicillin RS and USP Clavulanate Lithium RS in Medium at known concentrations similar to those expected in the Sample solution

Sample solution: Pass a portion of the solution under test through a suitable filter, and dilute with Medium, if necessary.

**Analysis**

Samples: Standard solution and Sample solution

Calculate the amounts of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) and clavulanic acid ( $C_8H_9NO_5$ ) dissolved.

**Tolerances**

Amoxicillin: The percentage of the labeled amount of amoxicillin ( $C_{16}H_{19}N_3O_5S$ ) dissolved at the times specified conforms to Table 2.

**Table 2**

Time (h)	Amount Dissolved (%)
1	50–70
3	65–90
5	NLT 85

Clavulanic acid: NLT 85% (Q) of the labeled amount of clavulanic acid ( $C_8H_9NO_5$ ) is dissolved in 45 min.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES****• ORGANIC IMPURITIES**

Buffer: 13.8 g/L of monobasic sodium phosphate in water adjusted with phosphoric acid to a pH of 4.2

Solution A: Methanol and Buffer (1:199)

Solution B: Methanol and Buffer (10:90)

Mobile phase: See Table 3.

**Table 3**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
8	70	30
13	70	30
13.01	40	60
18	40	60
18.01	100	0
25	100	0
30	100	0

[NOTE—These gradient elution times are established on an HPLC system with a dwell volume of approximately 5 mL.



The gradient elution times in Table 3 can be adjusted as necessary to achieve the separation described.]

**System suitability solution:** 0.4 mg/mL of USP Amoxicillin RS and 30 µg/mL of USP Amoxicillin Related Compound D RS in water. Store the solution at 4°.

**Standard solution:** 0.4 mg/mL of USP Amoxicillin RS in water. Store the solution at 4°, and inject within 24 h.

**Sample solution:** 1 mg/mL of amoxicillin and 62.5 µg/mL of clavulanic acid from finely powdered Tablets (NLT 2) in water. Stir for about 60 min. Store the solution at 4°, and use within 24 h.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 5-cm; 3-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**Autosampler temperature:** 4°

#### System suitability

**Samples:** System suitability solution and Standard solution

#### Suitability requirements

**Resolution:** NLT 1.25 between the amoxicillin and amoxicillin related compound D peaks at a relative retention time of 0.83, *System suitability solution*

**Tailing factor:** NMT 1.8 for the amoxicillin peak, *Standard solution*

**Relative standard deviation:** NMT 1.0% for the amoxicillin peak, *Standard solution*

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F_1 \times (1/F_2) \times 100$$

$r_U$  = response of each impurity from the *Sample solution*

$r_S$  = response of amoxicillin from the *Standard solution*

$C_S$  = concentration of USP Amoxicillin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amoxicillin in the *Sample solution* (mg/mL)

$P$  = potency of amoxicillin in USP Amoxicillin RS (µg/mg)

$F_1$  = conversion factor, 0.001 mg/µg

$F_2$  = relative response factor (see Table 4)

**Acceptance criteria:** See Table 4. The reporting limit is 0.003%.

Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound I (D-hydroxyphenyl-glycine) <sup>a,b</sup>	0.15	—	—
Amoxicillin related compound A (6-aminopenicillanic acid) <sup>a,c</sup>	0.30	—	—
Clavulanic acid	0.39	—	—
Amoxicillin related compound D (amoxicillin open ring) <sup>a,d,e</sup>	0.63	0.74	—
Amoxicillin related compound B (L-amoxicillin) <sup>a,f</sup>	0.78	—	—
Amoxicillin related compound D (amoxicillin open ring) <sup>d,e</sup>	0.83	0.74	2.5
Amoxicillin	1.0	—	—
Amoxicillin related compound G (D-hydroxyphenyl-glycylamoxicillin) <sup>a,g</sup>	2.57	—	—
Amoxicillin related compound E (amoxicillin penilloic derivatives) <sup>a,h,i</sup>	2.63	—	—
	3.00		
Amoxicillin related compound C (amoxicillin rearrangement product) <sup>j</sup>	3.22	1.1	2.5
Amoxicillin open ring methyl ester <sup>a,k</sup>	3.38	—	—

<sup>a</sup> These are synthetic process impurities, which are controlled in the drug substance. They are listed here for reference only and are not to be reported.

<sup>b</sup> (R)-2-Amino-2-(4-hydroxyphenyl)acetic acid.

<sup>c</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid.

<sup>d</sup> The chromatographic system resolves two isomers of amoxicillin open ring.

<sup>e</sup> (4S)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxymethyl)-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>f</sup> (2S,5R,6R)-6-[(S)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>g</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>h</sup> The chromatographic system resolves two amoxicillin penilloic derivatives.

<sup>i</sup> (4S)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>j</sup> (4S)-2-[5-(4-Hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>k</sup> (4S)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]methoxy-carbonylmethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>l</sup> (2S,5R,6R)-6-[(2R)-2-[2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido]-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.



Table 4 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound J (amoxicillin open ring dimer) <sup>a</sup>	4.07	1.0	4.5
Any individual unspecified impurity	—	—	0.5

<sup>a</sup> These are synthetic process impurities, which are controlled in the drug substance. They are listed here for reference only and are not to be reported.

<sup>b</sup> (R)-2-Amino-2-(4-hydroxyphenyl)acetic acid.

<sup>c</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>d</sup> The chromatographic system resolves two isomers of amoxicillin open ring.

<sup>e</sup> (4S)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxymethyl)-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>f</sup> (2S,5R,6R)-6-[(S)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>g</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>h</sup> The chromatographic system resolves two amoxicillin penilloic derivatives.

<sup>i</sup> (4S)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]methyl-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>j</sup> (4S)-2-[5-(4-Hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>k</sup> (4S)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]methoxy-carbonylmethyl-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>l</sup> (2S,5R,6R)-6-[(2R)-2-[2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido]-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

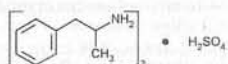
## SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed  $10^3$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g.

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.
- LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- USP REFERENCE STANDARDS (11)**
  - USP Amoxicillin RS
  - USP Amoxicillin Related Compound D RS
  - (4S)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamid-o](carboxymethyl)-5,5-dimethylthiazolidine-4-carboxylic acid.
  - $C_{16}H_{21}N_3O_6S$  383.42
  - USP Clavulanate Lithium RS

## Amphetamine Sulfate



$(C_9H_{13}N)_2 \cdot H_2SO_4$  368.49  
Benzeneethanamine,  $\alpha$ -methyl-, sulfate (2:1), ( $\pm$ ); ( $\pm$ )- $\alpha$ -Methylphenethylamine sulfate (2:1) [60-13-9].

## DEFINITION

Amphetamine Sulfate contains NLT 98.0% and NMT 102.0% of  $(C_9H_{13}N)_2 \cdot H_2SO_4$  on the dried basis.

## IDENTIFICATION

- A. INFRARED ABSORPTION (197M)**
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- C. IDENTIFICATION TESTS—GENERAL, Sulfate (191):** Meets the requirements  
*Sample solution:* 100 mg/mL

## ASSAY

### PROCEDURE

**Solution A:** Add 5.0 mL of trifluoroacetic acid to 900 mL of water, adjust with ammonium hydroxide to a pH of  $2.2 \pm 0.1$ , and add 100 mL of acetonitrile.

**Solution B:** Use degassed acetonitrile.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	65	35
20	0	100
22	0	100
23	100	0
30	100	0

**Standard solution:** 2.0 mg/mL of USP Dextroamphetamine Sulfate RS in *Solution A*

**System suitability solution:** Transfer 40 mL of the *Standard solution* to a 50-mL volumetric flask. Using a microliter syringe, add 1  $\mu$ L each of USP Dextroamphetamine Related Compound A RS and USP Dextroamphetamine Related Compound B RS. Dilute with *Standard solution* to volume, and mix.

**Sample solution:** 2.0 mg/mL of Amphetamine Sulfate in *Solution A*

### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 257 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—Identify the peaks by the relative retention times in *Impurity Table 1* under *Organic Impurities*. Amphetamine and dextroamphetamine have exactly the same retention time.]

### Suitability requirements

**Resolution:** NLT 3.0 between dextroamphetamine related compound A and dextroamphetamine related compound B, *System suitability solution*

**Tailing factor:** NMT 3.0, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $(C_9H_{13}N)_2 \cdot H_2SO_4$  in the portion of Amphetamine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for amphetamine sulfate from the *Sample solution*

$r_S$  = peak response for dextroamphetamine sulfate from the *Standard solution*

$C_S$  = concentration of USP Dextroamphetamine Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Amphetamine Sulfate in the *Sample solution* (mg/mL)



Acceptance criteria: 98.0%–102.0% on the dried basis

## IMPURITIES

### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.2%

### Organic Impurities

#### • PROCEDURE

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

#### Analysis

Samples: Standard solution and Sample solution

[NOTE—Identify the impurities by the relative retention times in Impurity Table 1.]

Calculate the percentage of each impurity in the portion of Amphetamine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each impurity from the Sample solution

$r_S$  = peak response for dextroamphetamine from the Standard solution

$C_S$  = concentration of USP Dextroamphetamine Sulfate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Amphetamine Sulfate in the Sample solution (mg/mL)

$F$  = relative response factor (see Impurity Table 1)

#### Acceptance criteria

Individual impurities: See Impurity Table 1.

Total impurities: NMT 1.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cathinone	0.81	55.6	0.25
Amphetamine	1.0	1.0	—
Benzaldehyde	1.73	105.3	0.25
Dextroamphetamine related compound A	1.88	1.5	0.25
Dextroamphetamine related compound B	2.05	1.8	0.25
Individual unspecified impurity	—	1.0	0.1

## SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample at 105° for 2 h: it loses NMT 1.0% of its weight.
- **DEXTROAMPHETAMINE**: A solution (20 mg/mL) is optically inactive.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)
  - USP Dextroamphetamine Sulfate RS
  - USP Dextroamphetamine Related Compound A RS
  - 1-Phenyl-2-propanol,  $C_9H_{12}O$  136.20 [CAS-14898-87-4].
  - USP Dextroamphetamine Related Compound B RS
  - Phenyl acetone,  $C_9H_{10}O$  134.18 [CAS-103-79-7].

## Amphetamine Sulfate Tablets

### DEFINITION

Amphetamine Sulfate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of amphetamine sulfate  $[(C_9H_{13}N)_2 \cdot H_2SO_4]$ .

### IDENTIFICATION

- **A. MELTING RANGE OR TEMPERATURE**, Class I (741)

Sample: Macerate a quantity of powdered Tablets, equivalent to 50 mg of amphetamine sulfate, with 10 mL of water for 30 min, and filter into a small flask. To the filtrate add 3 mL of 1 N sodium hydroxide. Cool to 10°–15°, add 1 mL of a mixture of absolute ether and benzoyl chloride (2:1), insert the stopper, and shake well for 3 min. Filter the precipitate, wash with 15 mL of cold water, and recrystallize twice from diluted alcohol. Dry the residue at 80° for 2 h.

Acceptance criteria: The crystals of the benzoyl derivative of amphetamine melt between 131° and 135°.

- **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

Diluted acetic acid: 14 mL of glacial acetic acid in 100 mL of water

Mobile phase: Dissolve 1.1 g of sodium 1-heptanesulfonate in 525 mL of water. Add 25 mL of diluted acetic acid and 450 mL of methanol. Adjust with glacial acetic acid to a pH of  $3.3 \pm 0.1$ . Pass through a 0.5- $\mu$ m membrane filter.

Standard solution: 0.3 mg/mL of USP Dextroamphetamine Sulfate RS in 0.12 N phosphoric acid

Sample solution: Nominally 0.3 mg/mL of amphetamine sulfate from NLT 20 finely powdered Tablets prepared as follows. Transfer a suitable amount of the powdered tablets to a suitable volumetric flask. Add 80% of the flask volume of 0.12 N phosphoric acid, and sonicate for 15 min. Dilute with 0.12 N phosphoric acid to volume. Pass through a 0.5- $\mu$ m membrane filter, discarding the first 20 mL of the filtrate.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm  $\times$  30-cm; 10- $\mu$ m packing L1

Flow rate: 2 mL/min. [NOTE—A 4.6-mm  $\times$  25-cm column; 5- $\mu$ m packing L1 may be used with a flow rate of 1 mL/min.]

Injection size: 50  $\mu$ L

#### System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 3

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of amphetamine sulfate  $[(C_9H_{13}N)_2 \cdot H_2SO_4]$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Dextroamphetamine Sulfate RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of amphetamine sulfate in the Sample solution (mg/mL)



Acceptance criteria: 93.0%–107.0%

## PERFORMANCE TESTS

### • DISSOLUTION, Procedure for a Pooled Sample (711)

Medium: Water; 500 mL

Apparatus 1: 100 rpm

Time: 45 min

**Standard solution:** USP Dextroamphetamine Sulfate RS in Medium having a known concentration of USP Dextroamphetamine Sulfate RS similar to the concentration expected in the sample.

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Diluted acetic acid:** 14 mL of glacial acetic acid in 100 mL of water

**Mobile phase:** 1.1 g of sodium 1-heptanesulfonate in 575 mL of water. Add 25 mL of Diluted acetic acid and 400 mL of methanol. Adjust with glacial acetic acid to a pH of  $3.3 \pm 0.1$ .

### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm  $\times$  30-cm; packing L1

Flow rate: 1 mL/min

Injection size: 500  $\mu$ L

### System suitability

Sample: Standard solution

### Suitability requirements

Relative standard deviation: NMT 2.0%

### Analysis

Calculate the percentage of the labeled amount of amphetamine sulfate  $[(C_9H_{13}N)_2 \cdot H_2SO_4]$  dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of the Standard solution (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of Medium, 500 mL

**Tolerances:** NLT 75% (Q) of amphetamine sulfate  $[(C_9H_{13}N)_2 \cdot H_2SO_4]$  is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in well-closed containers.

### • USP REFERENCE STANDARDS (11)

USP Dextroamphetamine Sulfate RS

9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid [1397-89-3].

» Amphotericin B has a potency of not less than 750  $\mu$ g of  $C_{47}H_{73}NO_{17}$  per mg, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, and store in a cold place.

**Labeling**—Label it to state whether it is intended for use in preparing dermatological and oral dosage forms or parenteral dosage forms.

### USP Reference standards (11)—

USP Amphotericin B RS

USP Nystatin RS

### Identification, Ultraviolet Absorption (197U)—

**Spectral range 1:** 240 to 320 nm.

**Solution 1:** prepared as directed for Test preparation in the Limit of amphotericin A, and compare its absorbance to that of the Amphotericin B standard preparation. An extra peak may occur at 304 nm in the spectrum of this solution.

**Spectral range 2:** 320 to 400 nm.

**Solution 2:** prepared as directed for Test preparation in the Limit of amphotericin A and then diluted with 9 volumes of methanol. Compare its absorbance to that of a similar dilution of the Amphotericin B standard preparation.

**Loss on drying (731)**—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

**Residue on ignition (281):** not more than 0.5%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid. [NOTE—Amphotericin B intended for use in preparing dermatological creams, lotions, and ointments, and oral suspensions and capsules, yields not more than 3.0%.]

### Limit of amphotericin A—

**Test preparation**—Dissolve about 50 mg of Amphotericin B, accurately weighed, in 10.0 mL of dimethyl sulfoxide in a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

**Nystatin standard preparation**—Dissolve about 20 mg of USP Nystatin RS, accurately weighed, in 40.0 mL of dimethyl sulfoxide in a 200-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

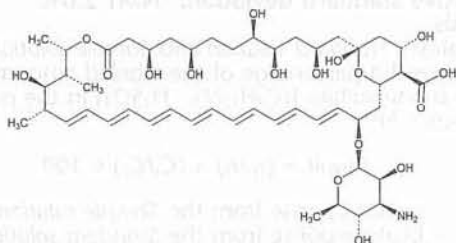
**Amphotericin B standard preparation**—Dissolve about 50 mg of USP Amphotericin B RS, accurately weighed, in 10.0 mL of dimethyl sulfoxide in a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Prepare this solution fresh daily.

**Procedure**—Concomitantly determine the absorbances of the Nystatin and Amphotericin B standard preparations and the Test preparation in 1-cm cells at 304 nm and at 282 nm, with a suitable spectrophotometer, using a 1 in 62.5 solution of dimethyl sulfoxide in methanol as the blank. Calculate the percentage of amphotericin A taken by the formula:

$$25W_N[(A_{B_{282}} \times A_{U_{304}}) - (A_{B_{304}} \times A_{U_{282}})] / [(A_{B_{282}} \times A_{N_{304}}) - (A_{B_{304}} \times A_{N_{282}})]W_U$$

in which  $W_N$  is the weight, in mg, of USP Nystatin RS taken,  $A_{B_{282}}$  and  $A_{B_{304}}$  are the absorbances of the Amphotericin B standard preparation at 282 nm and 304 nm, respectively,  $A_{N_{282}}$  and  $A_{N_{304}}$  are the absorbances of the Nystatin standard

## Amphotericin B



$C_{47}H_{73}NO_{17}$  924.08

Amphotericin B.

Amphotericin B.

[1R-(1R\*,3S\*,5R\*,6R\*,9R\*,11R\*,15S\*,16R\*,17R\*,18S\*,19E\*,21E,23E,25E,27E,29E,31E,33R\*,35S\*,36R\*,37S\*))]-33-[(3-Amino-3,6-dideoxy- $\beta$ -D-mannopyranosyl)oxy]-1,3,5,6,



preparation at 282 nm and 304 nm, respectively,  $A_{U282}$  and  $A_{U304}$  are the absorbances of the *Test preparation* at 282 nm and 304 nm, respectively, and  $W_U$  is the weight, in mg, of the Amphotericin B taken: not more than 5%, calculated on the dried basis, is found. [NOTE—Amphotericin B intended for use in preparing dermatological creams, lotions, and ointments, and oral suspensions and capsules, contains not more than 15% of amphotericin A, calculated on the dried basis.]

**Assay**—Proceed with amphotericin B as directed under *Antibiotics—Microbial Assays* (81).

## Amphotericin B Cream

» Amphotericin B Cream contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of Amphotericin B.

**Packaging and storage**—Preserve in collapsible tubes, or other well-closed containers.

**USP Reference standards** (11)—

USP Amphotericin B RS

**Minimum fill** (755): meets the requirements.

### Change to read:

**Assay**—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* (81), blending a suitable accurately weighed portion of Cream in a high-speed blender with a sufficient accurately measured volume of dimethyl sulfoxide to give a convenient concentration. Quantitatively dilute an accurately measured volume of this solution with dimethyl sulfoxide to obtain a stock solution having a concentration of about 20 µg of amphotericin B per mL. Quantitatively dilute an accurately measured volume of this stock solution with **Buffer B.10** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Amphotericin B for Injection

» Amphotericin B for Injection is a sterile complex of Amphotericin B and deoxycholate sodium and one or more suitable buffers. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{47}H_{73}NO_{17}$ .

### Change to read:

**Packaging and storage**—Preserve as described in **Packaging and Storage Requirements** (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017), in a refrigerator and protected from light.

**Labeling**—Label it to indicate that it is intended for use by intravenous infusion to hospitalized patients only, and that the solution should be protected from light during administration.

**USP Reference standards** (11)—

USP Amphotericin B RS

USP Endotoxin RS

**Bacterial Endotoxins Test** (85)—It contains not more than 5.0 USP Endotoxin Units per mg of amphotericin B. For products used or labeled for intrathecal injection, it contains not more than 0.9 USP Endotoxin Unit per mg of amphotericin B.

**Sterility Tests** (71)—It meets the requirements when tested as directed in the section *Membrane Filtration under Test for Sterility of the Product to be Examined*, 50 mg from each container being tested.

**pH** (791): between 7.2 and 8.0, in an aqueous solution containing 10 mg of amphotericin B per mL.

**Loss on drying** (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 8.0% of its weight.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

### Change to read:

#### Assay—

*Assay preparation 1* (where it is packaged as a single-dose container)—Constitute Amphotericin B for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively and stepwise with dimethyl sulfoxide to obtain a solution containing about 20 µg of amphotericin B per mL.

*Assay preparation 2* (where the labeling states the quantity of amphotericin B in a given volume of constituted solution)—Constitute Amphotericin B for Injection as directed in the labeling. Withdraw an accurately measured volume of the resultant solution, using a suitable hypodermic needle and syringe, and dilute quantitatively and stepwise with dimethyl sulfoxide to obtain a solution containing about 20 µg of amphotericin B per mL.

*Procedure*—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with **Buffer B.10** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Amphotericin B Lotion

» Amphotericin B Lotion contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of amphotericin B.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Amphotericin B RS

**Minimum fill** (755): meets the requirements.

**pH** (791): between 5.0 and 7.0.

### Change to read:

**Assay**—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* (81), quantitatively dissolving a suitable accurately measured volume of Lotion in sufficient dimethyl sulfoxide to give a convenient concentration. Quantitatively dilute an accurately measured volume of this solution with dimethyl sulfoxide to obtain a stock solution having a concentration of about 20 µg of amphotericin B per mL. Quantitatively dilute an accurately measured vol-



ume of this stock solution with **Buffer B.10** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Amphotericin B Ointment

» Amphotericin B Ointment is Amphotericin B in a suitable ointment base. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of amphotericin B.

**Packaging and storage**—Preserve in collapsible tubes, or other well-closed containers.

### USP Reference standards (11)—

USP Amphotericin B RS

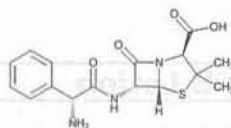
**Minimum fill** (755): meets the requirements.

**Water Determination, Method 1** (921): not more than 1.0%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

### Change to read:

**Assay**—Proceed as directed for amphotericin B under *Antibiotics—Microbial Assays* (81), using an accurately weighed portion of Ointment, equivalent to about 30 mg of amphotericin B, mixed with 10.0 mL of ether in a suitable glass-stoppered conical flask and allowed to stand, with intermittent shaking, for 1 hour. Add 20.0 mL of dimethyl sulfoxide and shake by mechanical means for 10 minutes. Dilute quantitatively and stepwise with dimethyl sulfoxide to a concentration of approximately 20 µg per mL. Quantitatively dilute an accurately measured volume of this stock solution with **Buffer B.10** (CN 1-May-2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Ampicillin



$C_{16}H_{19}N_3O_4S$  (anhydrous) 349.41  
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, [6-(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-, [2*S*-[2*α*,5*α*,6*β*(*S*<sup>\*</sup>)]-;  
(2*S*,5*R*,6*R*)-6-[(*R*)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid [69-53-4].  
Trihydrate 403.46  
[7177-48-2].

### DEFINITION

Ampicillin is anhydrous or contains three molecules of water of hydration. It contains NLT 900 µg/mg and NMT 1050 µg/mg of ampicillin ( $C_{16}H_{19}N_3O_4S$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **INFRARED ABSORPTION** (197K): Except that where the specimen under test is the trihydrate, both it and the USP Ampicillin Trihydrate RS are undried.

### ASSAY

#### • PROCEDURE

**Solution A:** 6.54 g/L of monobasic potassium phosphate and 0.34 g/L of dibasic potassium phosphate, adjusted with 1 N sodium hydroxide or 1 N phosphoric acid to a pH of 5.5 before final dilution

**Solution B:** Acetonitrile and *Solution A* (2:23)

**Solution C:** Acetonitrile and *Solution A* (3:7)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution B (%)	Solution C (%)
0	100	0
6	100	0
15	0	100
16	0	100
18	100	0
20	100	0

**Solution D:** 46.3 g/L of monobasic potassium phosphate and 27.8 g/L of dibasic potassium phosphate, adjusted with 1 N sodium hydroxide or 1 N phosphoric acid to a pH of 6.5 before final dilution

**System suitability solution:** 0.5 mg/mL of USP Ampicillin RS and 0.1 mg/mL of USP Amoxicillin RS in acetonitrile, water, and *Solution D* (4:91:5) prepared as follows. Dissolve first in a mixture of acetonitrile, water, and *Solution D* (4:30:5), sonicating if necessary, and dilute with water to volume.

**Standard solution:** 0.5 mg/mL of USP Ampicillin RS in acetonitrile, water, and *Solution D* (4:91:5) prepared as follows. Dissolve first in a mixture of acetonitrile, water, and *Solution D* (4:30:5), sonicating if necessary, and dilute with water to volume. Analyze immediately after preparation.

**Sample solution:** 0.5 mg/mL of Ampicillin in acetonitrile, water, and *Solution D* (4:91:5) prepared as follows. Dissolve first in a mixture of acetonitrile, water, and *Solution D* (4:30:5), sonicating if necessary, and dilute with water to volume. Analyze immediately after preparation.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 10 between ampicillin and amoxicillin, *System suitability solution*

**Tailing factor:** NMT 1.4 for ampicillin, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in each mg of Ampicillin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

- $r_U$  = peak response from the *Sample solution*
- $r_S$  = peak response from the *Standard solution*
- $C_S$  = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)
- $C_U$  = concentration of Ampicillin in the *Sample solution* (mg/mL)
- $P$  = potency of USP Ampicillin RS (µg/mg)



Acceptance criteria: 900–1050 µg/mg on the anhydrous basis

## IMPURITIES

### • ORGANIC IMPURITIES, PROCEDURE 1

*Organic Impurities, Procedure 1* is recommended when the impurity profile includes ampicillin thiazepine.

**Solution A, Solution B, Solution C, Mobile phase, Solution D, System suitability solution, Sample solution, and Chromatographic system:** Prepare as directed in the Assay.

**Standard stock solution:** Prepare as directed for the Standard solution in the Assay.

**Standard solution:** 0.005 mg/mL of ampicillin in Solution D and water (1:19) from Standard stock solution. Transfer an aliquot of the Standard stock solution to a suitable volumetric flask, add Solution D, using about 5% of the final volume, and dilute with water to volume. Analyze immediately after preparation.

**Sensitivity solution:** 0.5 µg/mL of ampicillin in Solution D and water (1:19) from the Standard solution. Transfer an aliquot of the Standard solution to a suitable volumetric flask, add Solution D, using about 5% of the final volume, and dilute with water to volume.

### System suitability

**Samples:** Sensitivity solution, System suitability solution, and Standard solution

### Suitability requirements

**Signal-to-noise ratio:** NLT 3, Sensitivity solution

**Resolution:** NLT 10 between ampicillin and amoxicillin, System suitability solution

**Tailing factor:** NMT 1.4 for ampicillin, System suitability solution

**Relative standard deviation:** NMT 10.0%, Standard solution

### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Ampicillin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response of each impurity from the Sample solution

$r_s$  = peak response of ampicillin from the Standard solution

$C_s$  = concentration of USP Ampicillin RS in the Standard solution (mg/mL)

$C_u$  = concentration of Ampicillin in the Sample solution (mg/mL)

$P$  = potency of USP Ampicillin RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine <sup>a</sup>	0.27	0.5
Amoxicillin related compound A (6-aminopenicillanic acid) <sup>b</sup>	0.31	0.5
Ampicilloic acid <sup>c</sup>	0.45	1.0
Ampicillin thiazepine analog <sup>d</sup>	0.65	0.3
Ampicillin	1.0	—
Ampicillin rearrangement product (isomer 1) <sup>e</sup>	1.8	0.4
Ampicillin rearrangement product (isomer 2) <sup>e</sup>	2.0	0.3
Ampicillin oligomer 2 <sup>f</sup>	2.2	0.6
D-Phenylglycylampicillin <sup>g</sup>	2.5	0.8
Ampicillin oligomer 1 (dimer) <sup>h</sup>	2.6	1.0
Ampicillin oligomer 1 (trimer) <sup>i</sup>	2.9	0.4
Any individual unspecified impurity	—	0.25
Total impurities	—	3.0

<sup>a</sup> (R)-2-Amino-2-phenylacetic acid.

<sup>b</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>c</sup> (4S)-2-[(R)-2-Amino-2-phenylacetamido](carboxymethyl)-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>d</sup> (S)-6-[(R)-2-Amino-2-phenylacetamido]-2,2-dimethyl-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine-3-carboxylic acid.

<sup>e</sup> (4S)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>f</sup> (4S)-2-[1-[(R)-2-Amino-2-phenylacetamido]-2-[(1R)-2-(carboxy[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino)-2-oxo-1-phenylethylamino]-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>g</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>h</sup> (2S,5R,6R)-6-[(2R)-2-[2-[(R)-2-Amino-2-phenylacetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>i</sup> (4S,4'S)-2'-[[(1R,7R,13R)-1-Amino-14-[(2S,5R,6R)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-penta-oxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl]bis(5,5-dimethylthiazolidine-4-carboxylic acid)]

### • ORGANIC IMPURITIES, PROCEDURE 2, DIMETHYLANILINE (223):

Meets the requirements

*Organic Impurities, Procedure 2* is recommended when dimethylaniline is used during the production of Ampicillin.

### • ORGANIC IMPURITIES, PROCEDURE 3

*Organic Impurities, Procedure 3* is recommended when the impurity profile includes phenylpyrazinol, pivaloyl phenylglycine, pivaloyl aminopenicillanic acid, diphenyldiketopiperazine, and open ring dimer.

**Solution A:** 4 g/L of monobasic sodium phosphate dihydrate adjusted with 1 N sodium hydroxide to a pH of 5.0

**Solution B:** Acetonitrile

**Mobile phase:** See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	98	2
20	90	10
40	85	15
50	80	20
55	75	25



Table 3 (Continued)

Time (min)	Solution A (%)	Solution B (%)
60	75	25
62	98	2
70	98	2

**Diluent:** Acetonitrile and *Solution A* (2:98)

**System suitability solution:** 1.5 mg/mL of USP Ampicillin System Suitability Mixture RS in *Diluent*

**Standard solution:** 15 µg/mL of USP Ampicillin RS in *Diluent*

**Sample solution:** 1.5 mg/mL of Ampicillin in *Diluent*. Store the sample in the refrigerator, and discard after 60 min.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L7

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**Autosampler temperature:** 4°

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between the pivaloyl phenylglycine and diphenyldiketopiperazine peaks, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ampicillin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response of each impurity from the *Sample solution*

$r_s$  = peak response of ampicillin from the *Standard solution*

$C_s$  = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Ampicillin in the *Sample solution* (mg/mL)

$P$  = potency of ampicillin in USP Ampicillin RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** See *Table 4* and *Table 5*. The limits in *Table 5* are to be used only where Ampicillin is intended for use in preparing veterinary products.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine <sup>a</sup>	0.15	1.0
Amoxicillin related compound A (6-aminopenicillanic acid) <sup>b</sup>	0.21	1.0
Ampicilloic acid <sup>c,d</sup>	0.40	1.0
	0.58	
L-Ampicillin <sup>e</sup>	0.65	1.0
Ampicillin	1.0	—
Ampilloic acid <sup>f,g</sup>	1.16	1.0
	1.40	
Ampicillin rearrangement product <sup>h,i</sup>	1.25	1.0
	1.48	
Phenylpyrazinol <sup>j</sup>	1.75	1.0
Pivaloyl phenylglycine <sup>k</sup>	1.87	1.0
Diphenyldiketopiperazine <sup>l</sup>	1.94	1.0
Ampicillin oligomer 2 <sup>m</sup>	2.08	1.0
D-Phenylglycylampicillin <sup>n</sup>	2.25	1.0
Pivaloyl aminopenicillanic acid <sup>o</sup>	2.54	1.0
	2.87	
	2.97	
Open ring dimer <sup>p,q</sup>	3.03	1.0
Ampicillin oligomer 1 (dimer) <sup>r</sup>	3.15	1.0
Ampicillinyl-D-phenylglycine <sup>s</sup>	3.86	1.0

<sup>a</sup> (R)-2-Amino-2-phenylacetic acid.

<sup>b</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>c</sup> (4S)-2-[[[(R)-2-Amino-2-phenylacetamido](carboxymethyl)-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>d</sup> The system resolves the two isomers of ampicilloic acid. The sum of the two isomers is reported.

<sup>e</sup> (2S,5R,6R)-6-[(S)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>f</sup> (4S)-2-[[[(R)-2-Amino-2-phenylacetamido](methyl)-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>g</sup> The system resolves the two isomers of ampilloic acid. The sum of the two isomers is reported.

<sup>h</sup> (4S)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>i</sup> The system resolves the two isomers of ampicillin rearrangement product. The sum of the two isomers is reported.

<sup>j</sup> 3-Phenylpyrazin-2-ol.

<sup>k</sup> (R)-2-Phenyl-2-pivalamidoacetic acid.

<sup>l</sup> 3,6-Diphenylpiperazine-2,5-dione.

<sup>m</sup> (4S)-2-[1-[(R)-2-Amino-2-phenylacetamido]-2-[(1R)-2-(carboxy[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino)-2-oxo-1-phenylethylamino]-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>n</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>o</sup> (2S,5R,6R)-3,3-Dimethyl-7-oxo-6-pivalamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>p</sup> (4S)-2-[1-[(R)-2-amino-2-phenylacetamido]-2-[(1R)-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino]-2-oxo-1-phenylethylamino]-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>q</sup> The system may resolve the three isomers of open ring dimer. The sum of the three isomers is reported.

<sup>r</sup> (2S,5R,6R)-6-[(2R)-2-[2-[(R)-2-Amino-2-phenylacetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>s</sup> (R)-2-[(2S,5R,6R)-6-[(R)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetic acid.

<sup>t</sup> (4S,4'S)-2,2'-[[[(1R,7R,13R)-1-Amino-14-[(2S,5R,6R)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-penta-oxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl]bis(5,5-dimethylthiazolidine-4-carboxylic acid)].



Table 4 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ampicillin oligomer 1 (trimer) <sup>a</sup>	4.19	1.0
Any individual unspecified impurity	—	0.10
Total impurities	—	5.0

<sup>a</sup> (R)-2-Amino-2-phenylacetic acid.<sup>b</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>c</sup> (4S)-2-[(R)-2-Amino-2-phenylacetamido](carboxymethyl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>d</sup> The system resolves the two isomers of ampicilloic acid. The sum of the two isomers is reported.<sup>e</sup> (2S,5R,6R)-6-[(S)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>f</sup> (4S)-2-[(R)-2-Amino-2-phenylacetamido]methyl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>g</sup> The system resolves the two isomers of ampicilloic acid. The sum of the two isomers is reported.<sup>h</sup> (4S)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>i</sup> The system resolves the two isomers of ampicillin rearrangement product. The sum of the two isomers is reported.<sup>j</sup> 3-Phenylpyrazin-2-ol.<sup>k</sup> (R)-2-Phenyl-2-pivalamidoacetic acid.<sup>l</sup> 3,6-Diphenylpiperazine-2,5-dione.<sup>m</sup> (4S)-2-[1-[(R)-2-Amino-2-phenylacetamido]-2-[(1R)-2-(carboxy[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino)-2-oxo-1-phenylethylamino]-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>n</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>o</sup> (2S,5R,6R)-3,3-Dimethyl-7-oxo-6-pivalamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>p</sup> (4S)-2-[1-[(R)-2-amino-2-phenylacetamido]-2-[(1R)-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino)-2-oxo-1-phenylethylamino]-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>q</sup> The system may resolve the three isomers of open ring dimer. The sum of the three isomers is reported.<sup>r</sup> (2S,5R,6R)-6-[(2R)-2-[(R)-2-Amino-2-phenylacetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>s</sup> (R)-2-[(2S,5R,6R)-6-[(R)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetic acid.<sup>t</sup> (4S,4'S)-2,2'-[(1R,7R,13R)-1-Amino-14-[(2S,5R,6R)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-pentaoxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl]bis(5,5-dimethylthiazolidine-4-carboxylic acid).

Where it is intended for use in preparing veterinary products:

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine <sup>a</sup>	0.15	2.0
Amoxicillin related compound A (6 aminopenicillanic acid) <sup>b</sup>	0.21	2.0
N-Formyl ampicilloic acid <sup>c</sup>	0.26	1.0
	0.40	
Ampicilloic acid <sup>d,e</sup>	0.58	2.0
L-Ampicillin <sup>f</sup>	0.65	2.0
Ampicillin	1.0	—
	1.16	
Ampicilloic acid <sup>g,h</sup>	1.40	2.0
Ampicillin rearrangement product <sup>i,j</sup>	1.25	
	1.48	2.0
Phenylpyrazinol <sup>k</sup>	1.75	2.0
Pivaloyl phenylglycine <sup>l</sup>	1.87	2.0
Diphenyldiketopiperazine <sup>m</sup>	1.94	2.0
Ampicillin oligomer 2 <sup>n</sup>	2.08	2.0
D-Phenylglycylampicillin <sup>o</sup>	2.25	2.0
Pivaloyl aminopenicillanic acid <sup>p</sup>	2.54	2.0
	2.87	0.50
	2.97	0.50
Open ring dimer <sup>q,r</sup>	3.03	0.50
Ampicillin oligomer 1 (dimer) <sup>s</sup>	3.15	4.5

<sup>a</sup> (R)-2-Amino-2-phenylacetic acid.<sup>b</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>c</sup> (4S)-2-[(R)-2-Amino-2-phenylacetamido](carboxymethyl)-3-formyl-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>d</sup> (4S)-2-[(R)-2-Amino-2-phenylacetamido](carboxymethyl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>e</sup> The system resolves the two isomers of ampicilloic acid. The sum of the two isomers is reported.<sup>f</sup> (2S,5R,6R)-6-[(S)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>g</sup> (4S)-2-[(R)-2-Amino-2-phenylacetamido]methyl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>h</sup> The system resolves the two isomers of ampicilloic acid. The sum of the two isomers is reported.<sup>i</sup> (4S)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>j</sup> The system resolves the two isomers of ampicillin rearrangement product. The sum of the two isomers is reported.<sup>k</sup> 3-Phenylpyrazin-2-ol.<sup>l</sup> (R)-2-Phenyl-2-pivalamidoacetic acid.<sup>m</sup> 3,6-Diphenylpiperazine-2,5-dione.<sup>n</sup> (4S)-2-[1-[(R)-2-Amino-2-phenylacetamido]-2-[(1R)-2-(carboxy[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino)-2-oxo-1-phenylethylamino]-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>o</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>p</sup> (2S,5R,6R)-3,3-Dimethyl-7-oxo-6-pivalamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>q</sup> (4S)-2-[1-[(R)-2-amino-2-phenylacetamido]-2-[(1R)-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino)-2-oxo-1-phenylethylamino]-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>r</sup> The system may resolve the three isomers of open ring dimer.<sup>s</sup> (2S,5R,6R)-6-[(2R)-2-[(R)-2-Amino-2-phenylacetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>t</sup> (R)-2-[(2S,5R,6R)-6-[(R)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetic acid.<sup>u</sup> (4S,4'S)-2,2'-[(1R,7R,13R)-1-Amino-14-[(2S,5R,6R)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-pentaoxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl]bis(5,5-dimethylthiazolidine-4-carboxylic acid).



Table 5 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ampicillinyl-D-phenylglycine <sup>a</sup>	3.86	2.0
Ampicillin oligomer 1 (trimer) <sup>u</sup>	4.19	2.0
Any individual unspecified impurity	—	0.5
Total impurities	—	5.0

<sup>a</sup> (R)-2-Amino-2-phenylacetic acid.<sup>b</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>c</sup> (4S)-2-[(R)-2-Amino-2-phenylacetamido](carboxymethyl)-3-formyl-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>d</sup> (4S)-2-[(R)-2-Amino-2-phenylacetamido](carboxymethyl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>e</sup> The system resolves the two isomers of ampicilloic acid. The sum of the two isomers is reported.<sup>f</sup> (2S,5R,6R)-6-[(S)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>g</sup> (4S)-2-[(R)-2-Amino-2-phenylacetamido](methyl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>h</sup> The system resolves the two isomers of ampicilloic acid. The sum of the two isomers is reported.<sup>i</sup> (4S)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>j</sup> The system resolves the two isomers of ampicillin rearrangement product. The sum of the two isomers is reported.<sup>k</sup> 3-Phenylpyrazin-2-ol.<sup>l</sup> (R)-2-Phenyl-2-pivalamidoacetic acid.<sup>m</sup> 3,6-Diphenylpiperazine-2,5-dione.<sup>n</sup> (4S)-2-[1-[(R)-2-Amino-2-phenylacetamido]-2-[(1R)-2-(carboxy[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino)-2-oxo-1-phenylethylamino]-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>o</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>p</sup> (2S,5R,6R)-3,3-Dimethyl-7-oxo-6-pivalamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>q</sup> (4S)-2-[1-[(R)-2-amino-2-phenylacetamido]-2-[(1R)-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino)-2-oxo-1-phenylethylamino]-2-oxoethyl]-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>r</sup> The system may resolve the three isomers of open ring dimer.<sup>s</sup> (2S,5R,6R)-6-[(2R)-2-[(R)-2-Amino-2-phenylacetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>t</sup> (R)-2-[(2S,5R,6R)-6-[(R)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetic acid.<sup>u</sup> (4S,4'S)-2,2'-[(1R,7R,13R)-1-Amino-14-[(2S,5R,6R)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-pentaoxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl]bis(5,5-dimethylthiazolidine-4-carboxylic acid).**• ORGANIC IMPURITIES, PROCEDURE 4**

Organic Impurities, Procedure 4 is recommended when the impurity profile includes ampicilloyl aminopenicillanic acid and penicillanyl ampicillinamide.

**Solution A:** 3.4 g/L of dibasic sodium phosphate dodecahydrate and 1.4 g/L of monobasic potassium phosphate adjusted with phosphoric acid to a pH of 5.5

**Solution B:** Acetonitrile

**Mobile phase:** See Table 6.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	99	1
1.5	95	5
6.5	90	10
7.5	89	11
13.5	84	16
16.5	75	25
18	60	40
25	99	1

**Standard solution:** 30 µg/mL of USP Amoxicillin Related Compound A RS, 30 µg/mL of D-phenylglycine, and 25 µg/mL of USP Ampicillin RS in *Solution A*

**Sample solution:** 2.5 mg/mL of Ampicillin in *Solution A*. Store the *Sample solution* in the refrigerator, and use within 9 h.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.0-mm × 15-cm; 3-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.3 mL/min

**Injection volume:** 5 µL

**Autosampler temperature:** 4°

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between D-phenylglycine and amoxicillin related compound A

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ampicillin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response of each impurity from the *Sample solution*

$r_s$  = peak response of ampicillin from the *Standard solution*

$C_s$  = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Ampicillin in the *Sample solution* (mg/mL)

$P$  = potency of ampicillin in USP Ampicillin RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** See Table 7. Disregard any peak with an area less than 0.03 times the area of the ampicillin peak in the *System suitability solution*.



Table 7

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine <sup>a</sup>	0.21	0.5
Amoxicillin related compound A (6-aminopenicillanic acid) <sup>b</sup>	0.32	0.5
	0.46	1.0
Ampicilloic acid <sup>c</sup>	0.57	1.0
Ampicillin thiazepine analog <sup>d,e</sup>	0.72	—
L-Ampicillin <sup>f</sup>	0.84	0.5
Ampilloyl aminopenicillanic acid <sup>g</sup>	0.87	0.5
Ampicillin	1.00	—
	1.15	1.0
	1.34	1.0
Ampilloic acid <sup>h</sup>		
Ampicillin rearrangement product <sup>i</sup>	1.24	1.0
Pivaloyl phenylglycine <sup>e,j</sup>	1.47	—
Phenylpyrazinol <sup>e,k</sup>	1.84	—
Diphenyldiketopiperazine <sup>e,l</sup>	1.94	—
Pivaloyl aminopenicillanic acid <sup>e,m</sup>	1.95	—
D-Phenylglycylampicillin <sup>n</sup>	2.08	1.0
Ampicillin oligomer 1 (dimer) <sup>n</sup>	2.16	1.0
Penicillanyl ampicillinamide <sup>n</sup>	2.27	1.0
Ampicillinyl-D-phenylglycine <sup>n</sup>	2.64	1.0
Any individual unspecified impurity	—	1.0
Total impurities	—	5.0

<sup>a</sup> (R)-2-Amino-2-phenylacetic acid.<sup>b</sup> (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>c</sup> (4S)-2-[[[(R)-2-Amino-2-phenylacetamido](carboxymethyl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>d</sup> (S)-6-[(R)-2-Amino-2-phenylacetamido]-2,2-dimethyl-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine-3-carboxylic acid.<sup>e</sup> These impurities are listed for information only. They are not to be reported. They are not to be included in total impurities.<sup>f</sup> (2S,5R,6R)-6-[(S)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>g</sup> (2S,5R,6R)-6-[(R)-2-Amino-2-phenylacetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidine-2-yl]acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>h</sup> (4S)-2-[[[(R)-2-Amino-2-phenylacetamido]methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>i</sup> (4S)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.<sup>j</sup> (R)-2-Phenyl-2-pivalamidoacetic acid.<sup>k</sup> 3-Phenylpyrazin-2-ol.<sup>l</sup> 3,6-Diphenylpiperazine-2,5-dione.<sup>m</sup> (2S,5R,6R)-3,3-Dimethyl-7-oxo-6-pivalamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>n</sup> (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>o</sup> (2S,5R,6R)-6-[(2R)-2-[(R)-2-Amino-2-phenylacetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidine-2-yl]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>p</sup> (2S,5R,6R)-6-[(2S,5R,6R)-6-[(R)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.<sup>q</sup> (R)-2-[(2S,5R,6R)-6-[(R)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-2-phenylacetic acid.**SPECIFIC TESTS****• STERILITY TESTS (71)**

**Sample solution:** Dissolve 6 g in 800 mL of *Fluid D* containing sufficient sterile penicillinase to inactivate the ampicillin, and swirl the vessel until dissolution is complete before filtering.

**Acceptance criteria:** Where the label states that Ampicillin is sterile, it meets the requirements when

tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

**• CRYSTALLINITY (695):** Meets the requirements**• PH (791)**

**Sample solution:** 10 mg/mL

**Acceptance criteria:** 3.5–6.0

**• WATER DETERMINATION, Method I (921):** NMT 2.0% where it is labeled as Ampicillin (anhydrous); between 12.0% and 15.0% where it is labeled as Ampicillin (trihydrate)**• BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Ampicillin is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Unit/mg of ampicillin.**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight containers.**• LABELING:** Label to indicate whether it is anhydrous or is the trihydrate. Where the quantity of ampicillin is indicated in the labeling of any preparation containing Ampicillin, this shall be understood to be in terms of anhydrous ampicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S). Where it is intended for use in preparing injectable dosage forms, the label states that it is the trihydrate and that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

If a test for *Organic Impurities* other than *Procedures 1* and *2* is used, then the labeling states with which *Organic Impurities* test the article complies. Where it is intended for use in preparing veterinary products, the label so states.

**• USP REFERENCE STANDARDS (11)**

USP Amoxicillin RS

USP Amoxicillin Related Compound A RS

(2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

C<sub>16</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub> 266.29

USP Ampicillin RS

USP Ampicillin System Suitability Mixture RS

This is a mixture which contains ampicillin, pivaloyl phenylglycine [(R)-2-phenyl-2-pivalamidoacetic acid; C<sub>13</sub>H<sub>17</sub>NO<sub>3</sub>; 235.28], diphenyldiketopiperazine (3,6-diphenylpiperazine-2,5-dione; C<sub>16</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>; 266.29), and other related compounds.

USP Ampicillin Trihydrate RS

USP Endotoxin RS

**Ampicillin Boluses**

» Ampicillin Boluses contain an amount of ampicillin (as the trihydrate) equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin (C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>4</sub>S).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the Boluses to indicate that they are for veterinary use only.

**USP Reference standards (11)**—

USP Ampicillin RS

**Identification**—Powder 1 or more Boluses, and prepare a solution containing the equivalent of 10 mg of ampicillin per mL in a mixture of acetone and 0.1 N hydrochloric acid (4:1); the resulting solution responds to the *Identification* test under *Ampicillin Capsules*.

**Uniformity of dosage units (905):** meet the requirements.



**Loss on drying** (731): not more than 5.0%.

#### Assay—

**Standard preparation**—Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics* (425), using USP Ampicillin RS.

**Assay preparation**—Place not fewer than 5 Boluses in a high-speed glass blender jar containing an accurately measured volume of water, and blend for  $4 \pm 1$  minutes. Dilute an accurately measured volume of this stock solution quantitatively and stepwise with water to obtain an *Assay preparation* containing about 1.25 mg of ampicillin per mL.

**Procedure**—Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics* (425). Calculate the quantity, in mg, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in each Bolus taken by the formula:

$$(T/D)(F/2000)(B-I)$$

in which  $T$  is the labeled quantity, in mg, of ampicillin in each Bolus; and  $D$  is the concentration, in mg per mL, of ampicillin in the *Assay preparation* on the basis of the labeled quantity in each Bolus and the extent of dilution.

## Ampicillin Capsules

### DEFINITION

Ampicillin Capsules contain an amount of ampicillin (anhydrous or as the trihydrate) equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHY

**Diluent:** Acetone and 0.1 N hydrochloric acid (4:1)

**Standard solution:** 5 mg/mL of USP Ampicillin RS in Diluent

**Sample solution:** 5 mg/mL of ampicillin in Diluent from the contents of Capsules

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 2  $\mu$ L

**Developing solvent system:** Acetone, toluene, glacial acetic acid, and water (650:100:25:100)

**Spray reagent:** 3 mg/mL of ninhydrin in alcohol

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* to the plate, and develop the chromatogram using the *Developing solvent system*. When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to air-dry. Locate the spots on the plate by spraying lightly with *Spray reagent*, and dry at 90° for 15 min.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

### ASSAY

#### • PROCEDURE

**Standard solution:** Prepare as directed for *Standard Preparation* in *Iodometric Assay—Antibiotics* (425), using USP Ampicillin RS.

**Sample solution:** Nominally 1.25 mg/mL of ampicillin prepared as follows. Place NLT 5 Capsules in a high-speed glass blender jar containing a suitable volume of water, and blend for  $4 \pm 1$  min. Dilute a suitable aliquot with water.

**Analysis:** Proceed as directed for *Procedure* in *Iodometric Assay—Antibiotics* (425).

Calculate the percentage of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in the portion of Capsules taken:

$$\text{Result} = (B - I) \times (F_1/2) \times (1/C_U) \times F_2 \times 100$$

$B$  = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

$I$  = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation and Titration* of the *Sample solution* (mL)

$F_1$  = factor as calculated in *Iodometric Assay—Antibiotics* (425)

$C_U$  = nominal concentration of ampicillin in the *Sample solution* (mg/mL)

$F_2$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–120.0%

### PERFORMANCE TESTS

#### • DISSOLUTION, *Procedure for a Pooled Sample* (711)

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Standard solution:**  $L/900$  mg/mL of USP Ampicillin RS in water, where  $L$  is the labeled amount of ampicillin in mg/Capsule

**Sample solution:** Use a filtered portion of the solution under test.

**Solution A:** 1 in 1000 solution of polyoxyethylene (23) lauryl ether in water

**Solution B:** Dissolve 20 g of hydroxylamine hydrochloride in 5 mL of *Solution A*, and add water to make 1000 mL.

**Buffer:** 26 mg/mL of sodium hydroxide and 3.1 mg/mL of sodium acetate in water

**Ferric nitrate solution:** Suspend 233 g of ferric nitrate in about 600 mL of water, add 2.8 mL of sulfuric acid, stir until the ferric nitrate is dissolved, add 1 mL of polyoxyethylene (23) lauryl ether, dilute with water to 1000 mL, and mix.

**Apparatus:** Automatic analyzer consisting of (1) a liquid sampler, (2) a proportioning pump, (3) suitable spectrophotometers equipped with matched flow cells and analysis capability at 480 nm, (4) a means of recording spectrophotometric readings, and/or computer for data retrieval and calculation, and (5) a manifold consisting of the components illustrated in *Figure 1*.



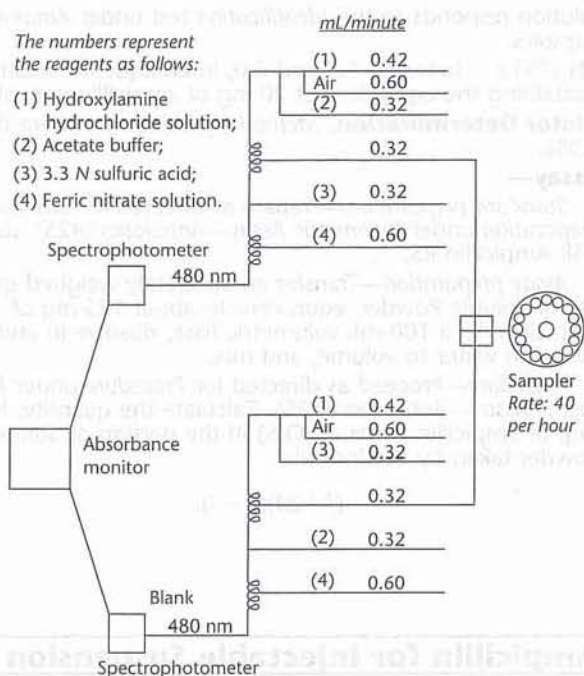


Figure 1

**Analysis:** With the sample line pumping water, the other lines pumping their respective reagents, and the spectrophotometer set at 480 nm, standardize the system until a steady absorbance baseline has been established. Transfer portions of the *Standard solution* and the *Sample solution* to sampler cups, and place in the sampler. Start the sampler, and conduct determinations of the *Standard solution* and the *Sample solution* typically at the rate of 40/h using a ratio of about 2:1 for sample and wash time.

Calculate the percentage of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times P \times F \times (1/L) \times 100$$

- $A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 900 mL  
 $P$  = potency of ampicillin in USP Ampicillin RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
 $L$  = label claim (mg/Capsule)

**Tolerances:** NLT 75% (Q) of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### SPECIFIC TESTS

- **WATER DETERMINATION, Method I** (921): NMT 4.0% where the Capsules contain anhydrous ampicillin, or between 10.0% and 15.0% where the Capsules contain ampicillin trihydrate

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label the Capsules to indicate whether the ampicillin therein is in the anhydrous form or is the trihydrate.

- **USP REFERENCE STANDARDS** (11)  
USP Ampicillin RS

## Ampicillin for Injection

### DEFINITION

Ampicillin for Injection contains an amount of Ampicillin Sodium equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ).

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, water, 1 M monobasic potassium phosphate, and 1 N acetic acid (80:909:10:1)

**Diluent:** Water, 1 M monobasic potassium phosphate, and 1 N acetic acid (989:10:1)

**Standard solution:** 1 mg/mL of USP Ampicillin RS in *Diluent*. Shake and sonicate, if necessary, to dissolve. Use this solution promptly after preparation.

**System suitability solution:** 0.12 mg/mL of caffeine in the *Standard solution*

**Sample solution 1** (where it is represented as being in a single-dose container): 1 mg/mL of ampicillin in *Diluent*. Constitute Ampicillin for Injection in a volume of *Diluent*, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with *Diluent*. Use this solution promptly after preparation.

**Sample solution 2** (where the label states the quantity of ampicillin in a given volume of constituted solution): 1 mg/mL of ampicillin in *Diluent*. Constitute 1 container of Ampicillin for Injection in a volume of *Diluent*, corresponding to the volume of solvent specified in the labeling. Dilute a suitable aliquot of the constituted solution with *Diluent*. Use this solution promptly after preparation.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Columns**

**Precolumn:** 4-mm  $\times$  5-cm; 5- to 10- $\mu\text{m}$  packing L1

**Analytical:** 4-mm  $\times$  30-cm; 5- to 10- $\mu\text{m}$  packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu\text{L}$

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ampicillin and caffeine are 0.5 and 1.0, respectively, *System suitability solution*.]

#### Suitability requirements

**Resolution:** NLT 2.0 between caffeine and ampicillin, *System suitability solution*

**Tailing factor:** NMT 1.4, *Standard solution*

**Capacity factor:** NMT 2.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in the container or in the volume of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)



- $C_U$  = concentration of *Sample solution 1* or *Sample solution 2* (mg/mL)  
 $P$  = potency of ampicillin in USP Ampicillin RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

Where the test for *Uniformity of Dosage Units* has been performed using the *Procedure for content uniformity*, use the average of these determinations as the Assay value.

Acceptance criteria: 90.0%–115.0%

## PERFORMANCE TESTS

### • UNIFORMITY OF DOSAGE UNITS (905)

*Procedure for content uniformity*

**Analysis:** Perform the Assay on individual containers using *Sample solution 1* or *Sample solution 2*, or both, as appropriate.

Acceptance criteria: Meets the requirements

## SPECIFIC TESTS

- **CRYSTALLINITY (695):** Meets the requirements. Freeze-dried products are exempt from this requirement.
- **pH (791)**  
Sample solution: 10.0 mg/mL of ampicillin  
Acceptance criteria: 8.0–10.0
- **WATER DETERMINATION, Method I (921):** NMT 2.0%
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **STERILITY TESTS (71):** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.15 USP Endotoxin Units/mg of ampicillin
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products (1)*, *Specific Tests*, *Completeness and clarity of solutions*.
- **OTHER REQUIREMENTS:** It meets the requirements of the tests for *Identification in Ampicillin Sodium*. It also meets the requirements in *Labeling (7)*, *Labels and Labeling for Injectable Products*.

## ADDITIONAL REQUIREMENTS

### Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *•Packaging and Storage Requirements (659)*, *Injection Packaging, Packaging for constitution* (CN 1-May-2017). Protect the constituted solution from freezing.
- **USP REFERENCE STANDARDS (11)**  
USP Ampicillin RS  
USP Ampicillin Sodium RS  
USP Endotoxin RS

## Ampicillin Soluble Powder

» Ampicillin Soluble Powder is a dry mixture of Ampicillin (as the trihydrate) and one or more suitable diluents and stabilizing agents. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin ( $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$ ).

**Packaging and storage—**Preserve in tight containers.

**Labeling—**Label it to indicate that it is for veterinary use only.

### USP Reference standards (11)—

USP Ampicillin RS

**Identification—**Dissolve a quantity of it in a mixture of acetone and 0.1 N hydrochloric acid (4:1) to obtain a solution containing 10 mg of ampicillin per mL: the resulting

solution responds to the *Identification* test under *Ampicillin Capsules*.

**pH (791):** between 3.5 and 6.0, in an aqueous solution containing the equivalent of 20 mg of ampicillin per mL.

**Water Determination, Method I (921):** not more than 5.0%.

### Assay—

**Standard preparation—**Prepare as directed for *Standard Preparation under Iodometric Assay—Antibiotics (425)*, using USP Ampicillin RS.

**Assay preparation—**Transfer an accurately weighed quantity of Soluble Powder, equivalent to about 125 mg of ampicillin, to a 100-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

**Procedure—**Proceed as directed for *Procedure under Iodometric Assay—Antibiotics (425)*. Calculate the quantity, in mg, of ampicillin ( $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$ ) in the portion of Soluble Powder taken by the formula:

$$(F/20)(B - I).$$

## Ampicillin for Injectable Suspension

» Ampicillin for Injectable Suspension is a dry mixture of ampicillin trihydrate and one or more suitable buffers, preservatives, stabilizers, and suspending agents. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin ( $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$ ).

### Change to read:

**Packaging and storage—**Preserve as described in *•Packaging and Storage Requirements (659)*, *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

### USP Reference standards (11)—

USP Ampicillin RS

USP Endotoxin RS

**Identification—**Dissolve a quantity in a mixture of acetone and 0.1 N hydrochloric acid (4:1) to obtain a solution containing 5 mg of ampicillin per mL: the resulting solution responds to the *Identification* test under *Ampicillin Capsules*.

**Bacterial Endotoxins Test (85)—**It contains not more than 0.15 Endotoxin Unit per mg of ampicillin.

**pH (791):** between 5.0 and 7.0, in the suspension constituted as directed in the labeling.

**Water Determination, Method I (921):** between 11.4% and 14.0%.

**Sterility Tests (71)—**It meets the requirements when tested as directed for *Antibiotic Solids, Bults, and Blends* in the section *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use *Fluid D*, to which has been added sufficient sterile penicillinase to inactivate the ampicillin and to swirl the vessel until solution is complete before filtering. If it does not dissolve completely, proceed as directed for *Solids* in the section *Direct Inoculation of the Culture Medium* under *Test for Sterility of the Product to be Examined*, except to use *Fluid Thioglycollate Medium* and *Soybean-Casein Digest Medium* containing sufficient penicillinase to inactivate the ampicillin in each vessel.

**Other requirements—**It meets the requirements for *Uniformity of Dosage Units (905)*, and for *Labeling (7)*, *Labels and Labeling for Injectable Products*.



**Assay—**

**Phosphate buffer solution**—Accurately weigh 68 g of monobasic potassium phosphate, and transfer to a 500-mL volumetric flask. Dissolve in and dilute with water to volume.

**Mobile phase**—Prepare a suitable mixture of water, acetonitrile, *Phosphate buffer solution*, and glacial acetic acid (3600:360:40:4). Pass through a 0.45- $\mu$ m nylon filter, and degas.

**Standard preparation**—Dissolve, with sonication, an accurately weighed quantity of USP Ampicillin RS in water to prepare a solution having 0.5 mg per mL. Pass through a 0.45- $\mu$ m PTFE filter, discarding the first 3 mL of the filtrate.

**Caffeine solution**—Transfer about 30 mg of caffeine, accurately weighed, to a 50-mL volumetric flask. Add 25 mL of water, sonicate to dissolve, and dilute with water to volume. Pass through a 0.45- $\mu$ m PTFE filter, discarding the first 3 mL of the filtrate.

**System suitability solution**—Prepare a solution of 1.0 mL of *Caffeine solution* and 9.0 mL of *Standard preparation*, and mix.

**Assay preparation**—Quantitatively dilute an accurately measured volume of Ampicillin for Injectable Suspension, constituted as directed in the labeling, with water to obtain a solution containing about 0.5 mg per mL. Pass through a 0.45- $\mu$ m PTFE filter, discarding the first 3 mL of the filtrate.

**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm analytical column that contains 10- $\mu$ m packing L1. The flow rate is about 2.0 mL per minute. The column temperature is maintained at 40°. Chromatograph the *System suitability solution* and the *Standard preparation*, and record the peak responses as directed for *Procedure*: the order of elution is ampicillin followed by caffeine; the resolution, *R*, between ampicillin and caffeine is greater than 2; the column efficiency is not less than 2000 theoretical plates for the ampicillin peak; the tailing factor is not greater than 1.4; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the quantity, in mg, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in each mL of the constituted solution of Ampicillin for Injectable Suspension taken by the formula:

$$CD(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ampicillin RS in the *Standard preparation*; *D* is the dilution factor used in preparing the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the average peak responses of the ampicillin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ampicillin for Oral Suspension

**DEFINITION**

Ampicillin for Oral Suspension contains an amount of Ampicillin (anhydrous or as the trihydrate) equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ), when constituted as directed. It contains one or more suitable buffers, colors, flavors, preservatives, and sweetening ingredients.

**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHY**

**Diluent**: Acetone and 0.1 N hydrochloric acid (4:1)

**Standard solution**: 5 mg/mL of USP Ampicillin RS in *Diluent*

**Sample solution**: Nominally 5 mg/mL of ampicillin in *Diluent*

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode**: TLC

**Adsorbent**: 0.25-mm layer of chromatographic silica gel mixture

**Application volume**: 2  $\mu$ L

**Developing solvent system**: Acetone, toluene, glacial acetic acid, and water (650:100:25:100)

**Spray reagent**: 3 mg/mL of ninhydrin in alcohol

**Analysis**

**Samples**: *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* to the plate, and develop the chromatogram in the *Developing solvent system*. When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to air-dry. Locate the spots on the plate by spraying lightly with *Spray reagent*, and dry at 90° for 15 min.

**Acceptance criteria**: The *R<sub>f</sub>* value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

**ASSAY**• **PROCEDURE**

**Standard solution**: Prepare as directed for *Standard preparation* in *Iodometric Assay—Antibiotics* <425>, using USP Ampicillin RS.

**Sample solution**: Nominally 1.25 mg/mL of ampicillin prepared as follows. Dilute a suitable aliquot of Ampicillin for Oral Suspension, constituted as directed in the labeling, freshly mixed and free from air bubbles, with water.

**Analysis**: Proceed as directed for *Iodometric Assay—Antibiotics* <425>, *Procedure*.

Calculate the percentage of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in the portion of Ampicillin for Oral Suspension taken:

$$\text{Result} = (B - I) \times F \times (1/C_U) \times 100$$

*B* = volume of 0.01 N sodium thiosulfate consumed in the *Blank Determination* (mL)

*I* = volume of 0.01 N sodium thiosulfate consumed in the *Inactivation and Titration* (mL)

*F* = factor as calculated in *Iodometric Assay—Antibiotics* <425>

*C<sub>U</sub>* = nominal concentration of ampicillin in the *Sample solution* (mg/mL)

**Acceptance criteria**: 90.0%–120.0%

**PERFORMANCE TESTS**

• **DELIVERABLE VOLUME** (698): Meets the requirements

• **UNIFORMITY OF DOSAGE UNITS** (905)

For single-unit containers

**Acceptance criteria**: Meets the requirements

**SPECIFIC TESTS**

• **pH** (791)

**Sample solution**: Constitute as directed in the labeling.

**Acceptance criteria**: 5.0–7.5

• **WATER DETERMINATION, Method I** (921): NMT 2.5% where the solid for Oral Suspension contains anhydrous ampicillin or NMT 5.0% if it contains ampicillin trihydrate and the equivalent of 100 mg/mL of ampicillin when constituted as directed in the labeling



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label to indicate whether the ampicillin therein is in the anhydrous form or is the trihydrate.
- **USP REFERENCE STANDARDS (11)**  
USP Ampicillin RS

**Ampicillin Tablets****DEFINITION**

Ampicillin Tablets contain an amount of Ampicillin (anhydrous form or trihydrate form) equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ).

**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHY**

**Diluent:** Acetone and 0.1 N hydrochloric acid (4:1)  
**Standard solution:** 5 mg/mL of USP Ampicillin RS in Diluent

**Sample solution:** 5 mg/mL of ampicillin from powdered Tablets in Diluent

**Chromatographic system**

(See Chromatography (621), Thin-Layer Chromatography.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 2  $\mu$ L

**Developing solvent system:** Acetone, toluene, glacial acetic acid, and water (650:100:25:100)

**Spray reagent:** 3 mg/mL of ninhydrin in alcohol

**Analysis**

**Samples:** Standard solution and Sample solution

Apply the Standard solution and the Sample solution to the plate, and develop the chromatogram using the Developing solvent system. When the solvent front has moved about three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to air-dry. Locate the spots on the plate by spraying lightly with the Spray reagent, and dry at 90° for 15 min.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the Sample solution corresponds to that of the Standard solution.

**ASSAY**• **PROCEDURE**

**Standard solution:** Prepare as directed for Standard Preparation in Iodometric Assay—Antibiotics (425), using USP Ampicillin RS.

**Sample solution:** Place NLT 5 Tablets in a high-speed glass blender jar containing an accurately measured volume of water, and blend for  $4 \pm 1$  min. Dilute a suitable aliquot with water to obtain a concentration of 1.25 mg/mL of ampicillin.

**Analysis**

**Samples:** Standard solution and Sample solution  
Proceed as directed for Procedure in Iodometric Assay—Antibiotics (425).

Calculate the percentage of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in the portion of Tablets taken:

$$\text{Result} = (B - I) \times (F_1/2) \times (1/C_U) \times F_2 \times 100$$

- $B$  = volume of 0.01 N sodium thiosulfate consumed in the Blank Determination (mL)  
 $I$  = volume of 0.01 N sodium thiosulfate consumed in the Inactivation and Titration of the Sample solution (mL)  
 $F_1$  = factor as calculated in Iodometric Assay—Antibiotics (425)

$C_U$  = nominal concentration of ampicillin in the Sample solution (mg/mL)

$F_2$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–120.0%

**PERFORMANCE TESTS**• **DISSOLUTION, Procedure for a Pooled Sample (711)**

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Standard solution:** L/900 mg/mL of USP Ampicillin RS in water, where L is the labeled amount of ampicillin in mg/Tablet

**Sample solution:** Use a filtered portion of the solution under test.

**Solution A:** 1 in 1000 solution of polyoxyethylene (23) lauryl ether in water

**Solution B:** Dissolve 20 g of hydroxylamine hydrochloride in 5 mL of Solution A, and add water to make 1000 mL.

**Buffer:** 26 mg/mL of sodium hydroxide and 3.1 mg/mL of sodium acetate in water

**Ferric nitrate solution:** Suspend 233 g of ferric nitrate in about 600 mL of water, add 2.8 mL of sulfuric acid, stir until the ferric nitrate is dissolved, add 1 mL of polyoxyethylene (23) lauryl ether, dilute with water to 1000 mL, and mix.

**Apparatus:** Automatic analyzer consisting of (1) a liquid sampler, (2) a proportioning pump, (3) suitable spectrophotometers equipped with matched flow cells and analysis capability at 480 nm, (4) a means of recording spectrophotometric readings, and/or computer for data retrieval and calculation, and (5) a manifold consisting of the components illustrated in Figure 1.

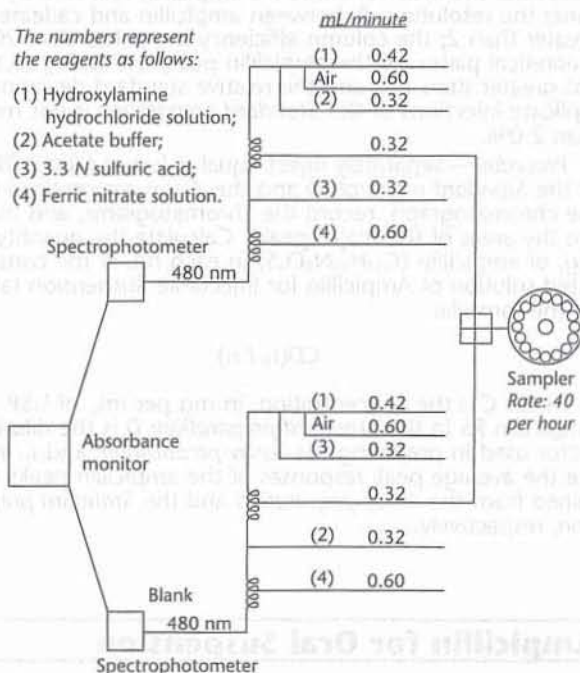


Figure 1

**Analysis**

**Samples:** Standard solution and Sample solution

With the sample line pumping water, the other lines pumping their respective reagents, and the spectrophotometer set at 480 nm, standardize the system until a steady absorbance baseline has been established. Transfer portions of the Standard solution and the Sample solution to sampler cups, and place in the sampler. Start the sampler, and conduct determinations of the Standard solution and the Sample solution



typically at the rate of 40/h using a ratio of about 2:1 for sample and wash time.  
Calculate the percentage of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times P \times F \times (1/L) \times 100$$

- $A_U$  = response of the *Sample solution*  
 $A_S$  = response of the *Standard solution*  
 $C_S$  = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)  
 $V$  = volume of medium, 900 mL  
 $P$  = potency of ampicillin in USP Ampicillin RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
 $L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921)**

Type of Tablets	Form of Ampicillin	Limit (%)
Nonchewable	Anhydrous	NMT 4.0
Nonchewable	Trihydrate	9.5–12.0
Chewable	Anhydrous	NMT 3.0
Chewable	Trihydrate	NMT 5.0
Tablets labeled for veterinary use only	Trihydrate	NMT 13.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** Label the Tablets to indicate whether the ampicillin therein is in the anhydrous form or is the trihydrate. Label chewable Tablets to indicate that they are to be chewed before swallowing. Tablets intended for veterinary use only are so labeled.
- **USP REFERENCE STANDARDS (11)**  
USP Ampicillin RS

### Ampicillin and Probenecid for Oral Suspension

» Ampicillin and Probenecid for Oral Suspension contains an amount of Ampicillin (as the trihydrate) equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of probenecid ( $C_{13}H_{19}NO_4S$ ). It contains one or more suitable colors, flavors, and suspending agents.

**Packaging and storage—**Preserve in tight, unit-dose containers.

#### USP Reference standards (11)—

USP Ampicillin RS  
USP Probenecid RS

#### Uniformity of dosage units (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements for *Content Uniformity* with respect to ampicillin and probenecid.

**Deliverable volume (698):** meets the requirements.

**pH (791):** between 5.0 and 7.5, in the suspension constituted as directed in the labeling.

**Water Determination, Method I (921):** not more than 5.0%.

#### Assay for ampicillin—

**Standard preparation—**Prepare as directed for *Standard Preparation* under *Iodometric Assay—Antibiotics (425)*, using USP Ampicillin RS.

**Assay preparation—**Constitute Ampicillin and Probenecid for Oral Suspension as directed in the labeling, and mix. Transfer the resulting suspension to a high-speed glass blender jar containing sufficient water to make 500.0 mL, and blend for about 10 minutes. Quantitatively dilute an accurately measured volume of this stock solution with water to obtain an *Assay preparation* containing about 1.25 mg of ampicillin per mL.

**Procedure—**Proceed as directed for *Procedure* under *Iodometric Assay—Antibiotics (425)*. Calculate the quantity, in mg, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) in the Ampicillin and Probenecid for Oral Suspension taken by the formula:

$$(L/D)(F/2000)(B-I)$$

in which  $L$  is the labeled quantity, in mg, of ampicillin in the Ampicillin and Probenecid for Oral Suspension; and  $D$  is the concentration, in mg per mL, of ampicillin in the *Assay preparation* on the basis of the labeled quantity in the Ampicillin and Probenecid for Oral Suspension and the extent of dilution.

#### Assay for probenecid—

**Standard preparation—**Dissolve an accurately weighed portion of USP Probenecid RS in sodium carbonate solution (1 in 100) to obtain a solution having a known concentration of about 1 mg per mL.

**Assay preparation—**Constitute Ampicillin and Probenecid for Oral Suspension as directed in the labeling, and mix. Quantitatively dilute the resulting suspension with sodium carbonate solution (1 in 100) to obtain a solution containing about 1 mg of probenecid per mL, mix, and filter.

**Procedure—**Transfer 2.0 mL of the clear *Assay preparation* to a 125-mL separator, and add 8.0 mL of 1.0 N hydrochloric acid. Extract this solution with four 20-mL portions of chloroform, filtering each extract through a glass wool pledget and 6 g of chloroform-washed anhydrous sodium sulfate into a 100-mL volumetric flask. Wash the pledget and the sodium sulfate with chloroform, collecting the washings in the 100-mL volumetric flask, dilute with chloroform to volume, and mix. Treat 2.0 mL of the *Standard preparation* in the same manner. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* at the wavelength of maximum absorbance at about 257 nm, with a suitable spectrophotometer, using chloroform washed with sodium carbonate solution (1 in 100) as the blank. Calculate the quantity, in mg, of probenecid ( $C_{13}H_{19}NO_4S$ ) in the Ampicillin and Probenecid for Oral Suspension taken by the formula:

$$C(L/D)(A_U/A_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Probenecid RS in the *Standard preparation*;  $L$  is the labeled quantity, in mg, of probenecid in the Ampicillin and Probenecid for Oral Suspension;  $D$  is the concentration, in mg per mL, of probenecid in the *Assay preparation* on the basis of the labeled quantity in the Ampicillin and Probenecid for Oral Suspension and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.



## Ampicillin Sodium

$C_{16}H_{18}N_3NaO_4S$  371.39

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, [6-(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-, monosodium salt, [2*S*-(2*α*,5*α*,6*β*(*S*\*))]-;

Monosodium *D*-(−)-6-(2-amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [69-52-3].

### DEFINITION

Ampicillin Sodium has a potency equivalent to NLT 845 μg and NMT 988 μg of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) per mg, calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B. IDENTIFICATION TESTS—GENERAL**, Sodium (191)

### ASSAY

#### • PROCEDURE

**Diluent:** Water, 1 M monobasic potassium phosphate, and 1 N acetic acid (989:10:1)

**Mobile phase:** Acetonitrile, water, 1 M monobasic potassium phosphate, and 1 N acetic acid (80:909:10:1)

**Standard solution:** 1 mg/mL of USP Ampicillin RS in *Diluent* using shaking and sonication, if necessary, to dissolve. Use this solution promptly after preparation.

**System suitability solution:** 0.12 mg/mL of caffeine in *Standard solution*

**Sample solution:** [NOTE—Ampicillin Sodium is hygroscopic. Minimize exposure to the atmosphere, and weigh promptly.] Equivalent to 1 mg/mL of anhydrous ampicillin in *Diluent*. [NOTE—Use this solution promptly after preparation.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column**

**Pre-column:** 4-mm × 5-cm; 5- to 10-μm packing L1

**Analytical column:** 4-mm × 30-cm; 5- to 10-μm packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20 μL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ampicillin and caffeine are 0.5 and 1.0, respectively, *System suitability solution*.]

#### Suitability requirements

**Resolution:** NLT 2.0 between the caffeine and the ampicillin peaks, *System suitability solution*

**Tailing factor:** NMT 1.4, *Standard solution*

**Capacity factor:** NMT 2.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in μg, of  $C_{16}H_{19}N_3O_4S$  in each mg of Ampicillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Ampicillin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Ampicillin Sodium in the *Sample solution* (mg/mL)

$P$  = potency of USP Ampicillin RS (μg/mg)

Acceptance criteria: 845–988 μg/mg on the anhydrous basis

### IMPURITIES

#### Organic Impurities

##### • PROCEDURE 1: LIMIT OF METHYLENE CHLORIDE

**Internal standard solution:** 2.1 mg/mL of dioxane in dimethyl sulfoxide

**Standard solution:** 0.33 mg/mL of methylene chloride in *Internal standard solution*

**Sample solution:** 166.7 mg/mL of Ampicillin Sodium in *Internal standard solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 1.8-m × 4-mm glass column packed with a 10% phase G39 on unsilanized support S1A

**Temperature**

**Column:** 65°

**Injector:** 100°

**Detector block:** 260°

**Carrier gas:** Nitrogen

**Flow rate:** 60 mL/min

**Injection size:** 1 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for methylene chloride and dioxane are 0.5 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 4 between methylene chloride and dioxane

**Relative standard deviation:** NMT 5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of methylene chloride in the portion of Ampicillin Sodium taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of methylene chloride to dioxane from the *Sample solution*

$R_S$  = peak response ratio of methylene chloride to dioxane from the *Standard solution*

$C_S$  = concentration of methylene chloride in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Ampicillin Sodium in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2%.

- **PROCEDURE 2: DIMETHYLANILINE** (223): Meets the requirement

### SPECIFIC TESTS

- **CRYSTALLINITY** (695): Meets the requirements. [NOTE—Ampicillin Sodium in the freeze-dried form is exempt from this requirement.]

- **PH** (791): 8.0–10.0

**Sample solution:** 10.0 mg/mL of ampicillin

- **WATER DETERMINATION**, *Method I* (921): NMT 2.0%

- **STERILITY TESTS** (71): Where the label states that Ampicillin Sodium is sterile, it meets the requirements.

- **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that Ampicillin Sodium is sterile or the label states that Ampicillin Sodium must be subjected to further processing during the processing of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Unit/mg of ampicillin.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.



• **USP REFERENCE STANDARDS** (11)

USP Ampicillin RS  
USP Ampicillin Sodium RS  
USP Endotoxin RS

## Ampicillin and Sulbactam for Injection

» Ampicillin and Sulbactam for Injection is a sterile, dry mixture of Ampicillin Sodium and Sulbactam Sodium. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) and sulbactam ( $C_8H_{11}NO_5S$ ), the labeled amounts representing proportions of ampicillin to sulbactam of 2:1. It contains not less than 563  $\mu$ g of ampicillin and 280  $\mu$ g of sulbactam per mg, calculated on the anhydrous basis.

### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

### USP Reference standards (11)—

USP Ampicillin RS  
USP Endotoxin RS  
USP Sulbactam RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.17 USP Endotoxin Unit in a portion equivalent to 1 mg of a mixture of ampicillin and sulbactam (0.67 and 0.33 mg, respectively).

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 8.0 and 10.0, in a solution containing 10 mg of ampicillin and 5 mg of sulbactam per mL.

**Water Determination, Method I** (921): not more than 2.0%.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

### Assay—

0.005 M Tetrabutylammonium hydroxide—Dilute 6.6 mL of a 40% solution of tetrabutylammonium hydroxide with water to obtain 1800 mL of solution. Adjust with 1 M phosphoric acid to a pH of  $5.0 \pm 0.1$ , dilute with water to 2000 mL, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of 0.005 M Tetrabutylammonium hydroxide and acetonitrile (1650:350). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Standard preparation*—Quantitatively dissolve accurately weighed quantities of USP Ampicillin RS and USP Sulbactam RS in *Mobile phase* to obtain a solution having known con-

centrations of about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]

*Resolution solution*—Prepare a solution of USP Sulbactam RS in 0.01 N sodium hydroxide containing 0.3 mg per mL, and allow to stand for 30 minutes. Adjust with phosphoric acid to a pH of  $5.0 \pm 0.1$ . Transfer 5 mL of the solution to a 25-mL volumetric flask, add 4.25 mL of acetonitrile, dilute with 0.005 M Tetrabutylammonium hydroxide to volume, and mix. Transfer 1 mL of this solution to a second 25-mL volumetric flask, add 15 mg of USP Ampicillin RS, dilute with *Mobile phase* to volume, and mix. [NOTE—Inject this solution promptly.]

*Assay preparation 1*—Mix the contents of a container of Ampicillin and Sulbactam for Injection. Quantitatively dissolve an accurately weighed portion of the powder in *Mobile phase* to obtain a solution having a concentration of about 1 mg of the powder per mL. [NOTE—Inject this solution promptly.]

*Assay preparation 2* (where it is represented as being in a single-dose container)—Constitute a container of Ampicillin and Sulbactam for Injection with a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw the total withdrawable contents from the container, using a suitable hypodermic needle and syringe, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]

*Assay preparation 3* (where the label states the quantities of ampicillin and sulbactam in a given volume of constituted solution)—Constitute a container of Ampicillin and Sulbactam for Injection with a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing about 0.6 mg of ampicillin per mL and 0.3 mg of sulbactam per mL. [NOTE—Inject this solution promptly.]

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4-mm  $\times$  30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times are about 0.7 for ampicillin and 1.0 for sulbactam alkaline degradation product; and the resolution,  $R$ , between ampicillin and sulbactam alkaline degradation product is not less than 4.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative retention times are about 0.35 for ampicillin and 1.0 for sulbactam; the column efficiency determined from the sulbactam peak is not less than 3500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the appropriate *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in  $\mu$ g, of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) and of sulbactam ( $C_8H_{11}NO_5S$ ) in the portion of Ampicillin and Sulbactam for Injection taken by the same formula:

$$(C_s P / C_u)(r_u / r_s)$$

in which  $C_s$  is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*;  $P$  is the assigned content, in  $\mu$ g per mg, of the appropriate USP Reference Standard;  $C_u$  is the concentration, in mg per mL, of Ampicillin and Sulbactam for Injection in *Assay preparation 1*, based on the weight, in mg, of powder removed from the container and the extent of dilution; and  $r_u$  and  $r_s$  are the peak areas for the appropriate analyte ob-

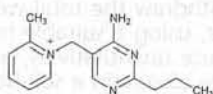


tained from *Assay preparation 1* and the *Standard preparation*, respectively. Calculate the quantities of ampicillin ( $C_{16}H_{19}N_3O_4S$ ) and of sulbactam ( $C_8H_{11}NO_5S$ ) withdrawn from the container, or in the volume of constituted solution taken by the same formula:

$$(L / D)(C_s P)(r_u / r_s)$$

in which *L* is the labeled quantity, in mg, of ampicillin or sulbactam, as appropriate, in the container or in the volume of constituted solution taken; *D* is the concentration, in mg per mL, of ampicillin or sulbactam in *Assay preparation 2* or *Assay preparation 3*, on the basis of the labeled quantity, in mg, of ampicillin or sulbactam, as appropriate, in the container and the extent of dilution; *r<sub>u</sub>* and *r<sub>s</sub>* are the peak areas for the appropriate analyte obtained from *Assay preparation 2* or *Assay preparation 3* and the *Standard preparation*, respectively; and the other terms are as defined above.

## Ampromium



Cl<sup>-</sup> · HCl

$C_{14}H_{19}ClN_4 \cdot HCl$  315.24  
1-[(4-Amino-2-propyl-5-pyrimidinyl)methyl]-2-methylpyridinium chloride monohydrochloride;  
1-[(4-Amino-2-propyl-5-pyrimidinyl)methyl]-2-picolinium chloride monohydrochloride [137-88-2].

### DEFINITION

Ampromium contains NLT 97.0% and NMT 101.0% of ampromium ( $C_{14}H_{19}ClN_4 \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)  
Sample: Previously dried  
Acceptance criteria: Meets the requirements
- **B. ULTRAVIOLET ABSORPTION** (197U)  
Analytical wavelength: 246 nm  
Sample solution: 10 µg/mL in 0.1 N hydrochloric acid  
Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

### ASSAY

- **PROCEDURE**  
Diluent: Methanol, acetonitrile, and water (45:5:50)  
Mobile phase: 6 g of sodium 1-heptanesulfonate in 500 mL of water. Add 12 mL of glacial acetic acid, 2.0 mL of triethylamine, 450 mL of methanol, and 50 mL of acetonitrile. Pass through a suitable filter of 0.5-µm or finer pore size.  
System suitability solution: 0.5 mg/mL of USP Ampromium RS and 0.2 mg/mL of 2-picoline in Diluent  
Standard solution: 0.5 mg/mL of USP Ampromium RS in Diluent  
Sample solution: 0.5 mg/mL of Ampromium in Diluent  
**Chromatographic system**  
(See Chromatography (621), System Suitability.)  
Mode: LC  
Detector: UV 254 nm  
Column: 4.6-mm × 25-cm; packing L13  
Flow rate: 0.6 mL/min  
Injection volume: 10 µL  
**System suitability**  
Sample: System suitability solution  
Suitability requirements  
Resolution: NLT 7 between ampromium and 2-picoline

**Column efficiency:** NLT 6500 theoretical plates from the ampromium peak

**Tailing factor:** NMT 2.3 for the ampromium peak

**Relative standard deviation:** NMT 1.0% for ampromium

### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of ampromium ( $C_{14}H_{19}ClN_4 \cdot HCl$ ) in the portion of Ampromium taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times 100$$

*r<sub>u</sub>* = peak response from the Sample solution

*r<sub>s</sub>* = peak response from the Standard solution

*C<sub>s</sub>* = concentration of USP Ampromium RS in the Standard solution (mg/mL)

*C<sub>u</sub>* = concentration of Ampromium in the Sample solution (mg/mL)

**Acceptance criteria:** 97.0%–101.0% on the dried basis

### SPECIFIC TESTS

#### • LOSS ON DRYING (731)

**Analysis:** Dry a sample at a pressure not exceeding 5 mm of mercury at 100° for 3 h.

**Acceptance criteria:** NMT 1.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** (11)  
USP Ampromium RS

## Ampromium Soluble Powder

### DEFINITION

Ampromium Soluble Powder contains NLT 95.0% and NMT 105.0% of the labeled amount of ampromium ( $C_{14}H_{19}ClN_4 \cdot HCl$ ).

### IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION** (197U)  
Sample solution: 10 µg/mL (filtered) in 0.1 N hydrochloric acid  
Acceptance criteria: Meets the requirements

### ASSAY

- **PROCEDURE**  
Diluent: Methanol, acetonitrile, and water (45:5:50)  
Mobile phase: 6 g of sodium 1-heptanesulfonate in 500 mL of water. Add 12 mL of glacial acetic acid, 2.0 mL of triethylamine, 450 mL of methanol, and 50 mL of acetonitrile. Pass through a suitable filter of 0.5-µm or finer pore size.  
System suitability solution: 0.5 mg/mL of USP Ampromium RS and 0.2 mg/mL of 2-picoline in Diluent  
Standard solution: 0.5 mg/mL of USP Ampromium RS in Diluent  
Sample solution: Nominally 0.5 mg/mL of ampromium in Diluent, prepared as follows. Transfer a portion of Soluble Powder, equivalent to 50 mg of ampromium, to a 100-mL volumetric flask, add 75 mL of Diluent, and sonicate for 10 min. Allow to cool to room temperature, and dilute with Diluent to volume. Pass through a suitable filter of 0.5-µm or finer pore size, and use the clear filtrate.  
**Chromatographic system**  
(See Chromatography (621), System Suitability.)



Mode: LC  
 Detector: UV 254 nm  
 Column: 4.6-mm × 25-cm; packing L13  
 Flow rate: 0.6 mL/min  
 Injection volume: 10 µL

#### System suitability

Sample: *System suitability solution*

#### Suitability requirements

Resolution: NLT 7 between amprolium and 2-picoline

Column efficiency: NLT 6500 theoretical plates from the amprolium peak

Tailing factor: NMT 2.3 for the amprolium peak

Relative standard deviation: NMT 1.0% for amprolium

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of amprolium ( $C_{14}H_{19}ClN_4 \cdot HCl$ ) in the portion of Soluble Powder taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Amprolium RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of amprolium in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS (11)**  
USP Amprolium RS

### Amprolium Oral Solution

» Amprolium Oral Solution contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of amprolium ( $C_{14}H_{19}ClN_4 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight containers, protected from light. Store at a temperature between 5° and 30°, in a dry place.

**Labeling**—Label it to indicate that it is for veterinary use only.

#### USP Reference standards (11)—

USP Amprolium RS

#### Identification, Ultraviolet Absorption (197U)—

*Solution:* 10 µg per mL, filtered.

*Medium:* 0.1 N hydrochloric acid.

**pH** (791): between 2.5 and 3.0.

#### Assay—

*Mobile phase*—To 4.5 g of sodium 1-hexanesulfonate add 1500 mL of water, 400 mL of methanol, and 100 mL of acetonitrile, mix, and allow to cool to room temperature. Adjust with phosphoric acid to a pH of 5.1, and pass through a filter having a 0.5-µm or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Quantitatively dissolve an accurately weighed quantity of USP Amprolium RS in water to obtain a solution having a known concentration of about 0.5 mg per mL.

*Assay preparation*—Transfer an accurately measured volume of Oral Solution, equivalent to about 960 mg of amprolium, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this stock solution to a second 100-mL volumetric flask, dilute with water to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 268-nm detector and a 3.9-mm × 30-cm column that contains packing L11. The column is maintained at a constant temperature of about 45°. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak areas for amprolium. Calculate the quantity, in mg, of amprolium ( $C_{14}H_{19}ClN_4 \cdot HCl$ ) in each mL of the Oral Solution taken by the formula:

$$(2000C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Amprolium RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the amprolium peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Amyl Nitrite

$C_5H_{11}NO_2$  117.15

Mixture of nitrous acid, 2-methylbutyl ester, and nitrous acid, 3-methylbutyl ester [8017-89-8; 110-46-3].

» Amyl Nitrite is a mixture of the nitrite esters of 3-methyl-1-butanol and 2-methyl-1-butanol. It contains not less than 85.0 percent and not more than 103.0 percent of  $C_5H_{11}NO_2$ .

**Caution**—Amyl Nitrite is very flammable. Do not use where it may be ignited.

**Packaging and storage**—Preserve in tight containers, and store in a cool place, protected from light.

#### USP Reference standards (11)—

USP Benzyl Benzoate RS

#### Identification—

**A:** The NMR spectrum recorded as directed in the *Assay* exhibits, among other peaks, a doublet with a band centered at about 1 ppm and a multiplet with a band centered at about 4.8 ppm representing methyl protons and methylene protons alpha to the nitrite group, respectively, both relative to the tetramethylsilane singlet at 0 ppm.

**B:** To a few drops of it add a mixture of 1 mL of ferrous sulfate TS and 5 mL of 3 N hydrochloric acid: a greenish brown color is produced.

**Specific gravity** (841): between 0.870 and 0.876.

**Acidity**—To 0.30 mL in a glass-stoppered cylinder add a mixture of 0.60 mL of 0.1 N sodium hydroxide, 10 mL of water, and 1 drop of phenolphthalein TS, and invert the cylinder three times: the red tint of the water layer is still perceptible.

**Limit of nonvolatile residue**—Allow 10 mL to evaporate at room temperature in a tared evaporating dish, in a well-ventilated hood, and dry the residue at 105° for 1 hour: the weight of the residue does not exceed 2 mg (0.02%).



**Content of total nitrites**—Inject a portion of Amyl Nitrite of suitable volume, but not more than 2  $\mu$ L, into a suitable gas chromatograph (see *Chromatography* (621)) equipped with a thermal conductivity detector. Under typical conditions, the instrument contains a 3-mm  $\times$  2-m column packed with a methyl polysiloxane oil, 25% by weight on suitable calcined diatomite, the column is maintained at about 80°, the injection port and detector block are maintained about 10° above the temperature of the column, and helium is used as a carrier gas at a flow rate of about 60 mL per minute. From the area under the curve, calculate the percentage (a/a) of total nitrites, represented by the area under the main peak of the chromatogram, in the Amyl Nitrite taken: not less than 97.0% is found.

#### Assay—

**Solvent:** carbon tetrachloride.

**Internal standard**—USP Benzyl Benzoate RS.

**Procedure**—Transfer 4 to 5 mEq of *Internal standard*, accurately weighed, to a semimicro sampling tube, add 2 to 3 mL of carbon tetrachloride, apply a sampling valve and septum,\* thereby sealing the tube, and determine the weight of the sealed assembly. Open the valve, introduce about 500  $\mu$ L of Amyl Nitrite with a syringe, close the valve, and determine the weight of the sealed assembly when it has attained constant weight. Shake the sampling tube and valve assembly, and transfer about 500  $\mu$ L of the solution to a precision NMR tube as directed for *Absolute Method of Quantitation* under *Nuclear Magnetic Resonance* (761). With no spinning, or with the spinning adjusted so that the spinning side bands of neither the substance under assay nor the *Internal standard* interfere with the regions to be integrated, record as  $A_5$  the average area of the *Internal standard* singlet appearing at about 5.3 ppm, representing the methylene protons of benzyl benzoate, and record as  $A_U$  the average area of the multiplet with a band center at about 4.8 ppm, representing the alpha methylene protons of amyl nitrite, with reference to the tetramethylsilane singlet at 0 ppm. Calculate the quantity of  $C_5H_{11}NO_2$  in the Amyl Nitrite taken, using 58.57 as the equivalent weight of amyl nitrite ( $EW_U$ ) and 106.12 as that of benzyl benzoate ( $EW_5$ ).

### Amyl Nitrite Inhalant

» Amyl Nitrite Inhalant contains a mixture of the nitrite esters of 3-methyl-1-butanol and 2-methyl-1-butanol. It contains not less than 80.0 percent and not more than 105.0 percent of  $C_5H_{11}NO_2$ . It contains a suitable stabilizer.

**Caution**—Amyl Nitrite Inhalant is very flammable. Do not use where it may be ignited.

**Packaging and storage**—Preserve in tight, unit-dose glass containers, wrapped loosely in gauze or other suitable material, and store in a cool place, protected from light.

**USP Reference standards** (11)—

USP Benzyl Benzoate RS

**Specific gravity** (841): between 0.870 and 0.880.

**Content of total nitrites**—Remove the gauze or other covering, place the glass container of Inhalant upright in a dry ice-acetone slurry, and cool for 10 minutes. Dry the container of Inhalant, place it in a pointed glass tube, and break the container with a glass rod. Proceed as directed for *Total nitrites* under *Amyl Nitrite*: not less than 95.0% is found.

\* Suitable sampling tubes, sampling valves, and septums are available, respectively, as catalog Nos. K-749000, K-749100, and K-749102 (50 septums) or K-749101 (100 septums), from Kontes Glass Company, Vineland, NJ 08360.

**Other requirements**—It responds to the *Identification* tests and meets the requirements of the test for *Acidity* under *Amyl Nitrite*.

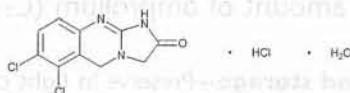
#### Assay—

**Solvent:** carbon tetrachloride.

**Internal standard**—USP Benzyl Benzoate RS.

**Procedure**—Remove the gauze or other covering from 1 or more Inhalant ampuls containing a total of 300 to 400  $\mu$ L of amyl nitrite. Weigh accurately the clean and dry intact glass ampul(s), and place the weighed specimen in a freezer for not less than 15 minutes. Transfer the chilled specimen to a glass-stoppered, 25-mL conical flask containing a solution of 4 to 5 mEq of *Internal standard*, accurately weighed, in 1 to 2 mL of carbon tetrachloride. Break the ampul(s) with a glass rod, and rinse any sample or glass fragments adhering to the glass rod with 1 mL of carbon tetrachloride into the main assay solution. Insert the stopper in the flask immediately, mix, and proceed as directed for *Absolute Method of Quantitation* under *Nuclear Magnetic Resonance* (761), beginning with "When dissolution has been completed." With no spinning, or with the spinning adjusted so that the spinning side bands of neither the substance under assay nor the *Internal standard* interfere with the regions to be integrated, record as  $A_5$  the average area of the *Internal standard* singlet appearing at about 5.3 ppm, representing the methylene protons of benzyl benzoate, and record as  $A_U$  the average area of the multiplet with a band center at about 4.8 ppm, representing the alpha methylene protons of amyl nitrite, with reference to the tetramethylsilane singlet at 0 ppm. Calculate the quantity of  $C_5H_{11}NO_2$  in the Inhalant taken, using 58.57 as the equivalent weight of amyl nitrite ( $EW_U$ ) and 106.12 as that of benzyl benzoate ( $EW_5$ ). Rinse the flask containing the assay preparation with three 5-mL portions of ether, decanting each rinsing carefully to avoid loss of glass fragments, and evaporate any remaining ether with the aid of a current of dry air. Transfer the dry glass fragments to a tared watch glass, weigh, and subtract the weight of the glass fragments from that of the intact ampul(s) to obtain the weight of the Inhalant taken.

### Anagrelide Hydrochloride



$C_{10}H_7Cl_2N_3O \cdot HCl \cdot H_2O$

Anhydrous

310.56

292.55

[58579-51-4].

Imidazo[2,1-*b*]quinazolin-2(3*H*)-one, 6,7-dichloro-1,5-dihydro-, monohydrochloride, monohydrate;  
6,7-Dichloro-1,5-dihydroimidazo[2,1-*b*]quinazolin-2(3*H*)-one monohydrochloride, monohydrate [823178-43-4].

#### DEFINITION

Anagrelide Hydrochloride contains NLT 98.0% and NMT 102.0% of anagrelide hydrochloride ( $C_{10}H_7Cl_2N_3O \cdot HCl$ ), calculated on the anhydrous basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements



**ASSAY****• PROCEDURE**

Use freshly prepared standard and sample solutions and inject within 2 h.

**Solution A:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** Acetonitrile and *Solution A* (1:3)

**Diluent:** Acetonitrile and water (1:1)

**Standard stock solution:** 0.5 mg/mL of anagrelide hydrochloride in acetonitrile prepared as follows. Transfer USP Anagrelide Hydrochloride RS into a suitable volumetric flask, add a small amount of 2 N hydrochloric acid (3 drops per every 50 mL of the final volume) and acetonitrile equivalent to fill about 80% of the final volume. Sonicate to dissolve, and dilute with acetonitrile to volume.

**Standard solution:** 0.05 mg/mL of anagrelide hydrochloride in *Diluent* from *Standard stock solution*

**Sample stock solution:** Weigh Anagrelide Hydrochloride, equivalent to 25 mg of anhydrous salt, into a 50-mL volumetric flask, add 3 drops of 2 N hydrochloric acid and 40 mL of acetonitrile. Sonicate to dissolve, and dilute with acetonitrile to volume.

**Sample solution:** Transfer 5 mL of *Sample stock solution* to a 50-mL volumetric flask, and dilute with *Diluent* to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 4-μm packing L11

**Flow rate:** 1.2 mL/min

**Injection volume:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 3000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of anagrelide hydrochloride ( $C_{10}H_7Cl_2N_3O \cdot HCl$ ) in the portion of Anagrelide Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of anagrelide from the *Sample solution*

$r_s$  = peak response of anagrelide from the *Standard solution*

$C_s$  = concentration of USP Anagrelide Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Anagrelide Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES**

**• RESIDUE ON IGNITION (281):** NMT 0.1%

**Delete the following:**

**• HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-

Jan-2018)

**• ORGANIC IMPURITIES**

Use freshly prepared standard and sample solutions and inject within 2 h.

**Mobile phase:** Proceed as directed in the Assay.

**Diluent A:** Use the *Diluent* as described in the Assay.

**Diluent B:** Acetonitrile and water (1:3)

**Standard stock solution A:** 0.05 mg/mL of USP Anagrelide Related Compound A RS in *Diluent A*

**Standard stock solution B:** 0.05 mg/mL of anagrelide related compound B in acetonitrile. Transfer USP Anagrelide Related Compound B RS into a suitable volumetric flask, add acetonitrile equivalent to fill about 50% of the final volume and a small amount of 2 N hydrochloric acid (3 drops per 200 mL of the final volume). Sonicate to dissolve, heat in the hot water bath if necessary, and dilute with acetonitrile to volume.

**Standard stock solution C:** 0.1 mg/mL of anagrelide hydrochloride in acetonitrile. Transfer USP Anagrelide Hydrochloride RS into a suitable volumetric flask, add acetonitrile equivalent to fill about 80% of the final volume and a small amount of 0.12 N hydrochloric acid (1 mL per 100 mL of the final volume). Sonicate to dissolve, and dilute with acetonitrile to volume.

**System suitability solution:** 0.25 μg/mL of each of anagrelide related compound A and anagrelide related compound B in *Mobile phase* from *Standard stock solution A* and *Standard stock solution B*

**Standard solution:** 0.05 μg/mL of anagrelide hydrochloride in *Mobile phase* from *Standard stock solution C*

**Sample stock solution:** Weigh Anagrelide Hydrochloride, equivalent to 25 mg of anhydrous salt, into a 50-mL volumetric flask. Add 45 mL of acetonitrile, sonicate, and swirl the flask until the preparation turns into a cloudy liquid. Add 1 drop of 0.12 N hydrochloric acid, swirl the flask until the liquid turns to clear, and dilute with acetonitrile to volume.

**Sample solution:** Transfer 5 mL of *Sample stock solution* into a 50-mL volumetric flask, and dilute with *Diluent B* to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 4-μm packing L11

**Autosampler temperature:** 5°

**Flow rate:** 1.2 mL/min

**Injection volume:** 50 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between anagrelide related compound B and anagrelide related compound A, *System suitability solution*

**Column efficiency:** NLT 3000 theoretical plates, *Standard solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 10.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Anagrelide Hydrochloride, on the anhydrous basis, taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of each impurity from the *Sample solution*

$r_s$  = peak response of anagrelide from the *Standard solution*

$C_s$  = concentration of USP Anagrelide Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Anagrelide Hydrochloride (anhydrous) in the *Sample solution* (mg/mL)

$F$  = relative response factor for each individual impurity (see *Table 1*)

**Acceptance criteria:** See *Table 1*. Disregard any impurity peak less than 0.05%.



Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Anagrelide related compound B <sup>a</sup>	0.40	0.43	0.3
Anagrelide related compound A <sup>b</sup>	0.55	0.37	0.15
Anagrelide open ring methyl ester (if present) <sup>c</sup>	0.80	0.51	0.25
Anagrelide	1.00	1.0	—
Anagrelide related compound C <sup>d</sup>	1.41	0.32	0.15
Anagrelide trichloro derivative <sup>e</sup>	2.44	1.0	0.15
Any unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.0

<sup>a</sup> (2-Amino-5,6-dichloroquinazolin-3(4*H*)-yl)acetic acid.<sup>b</sup> Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.<sup>c</sup> Methyl 2-(5,6-dichloro-2-imino-1,2-dihydroquinazolin-3(4*H*)-yl)acetate.<sup>d</sup> Ethyl 2-(5,6-dichloro-2-imino-1,2-dihydroquinazolin-3(4*H*)-yl)acetate hydrobromide.<sup>e</sup> 6,7,8-Trichloro-3,5-dihydroimidazo[2,1-*b*]quinazolin-2(1*H*)-one.**SPECIFIC TESTS**

- **WATER DETERMINATION, Method I (921):** 4.5%–7.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store in a cold place.
- **USP REFERENCE STANDARDS (11)**
  - USP Anagrelide Hydrochloride RS
  - USP Anagrelide Related Compound A RS
  - Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.  
C<sub>11</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub> 277.15
  - USP Anagrelide Related Compound B RS
  - (2-Amino-5,6-dichloroquinazolin-3(4*H*)-yl)acetic acid.  
C<sub>10</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub> 274.10

**Anagrelide Capsules****DEFINITION**

Anagrelide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of C<sub>10</sub>H<sub>7</sub>Cl<sub>2</sub>N<sub>3</sub>O.

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Solution A:** 1.0 g/L of sodium hexanesulfonate. Add 1.0 mL of phosphoric acid and filter.

**Mobile phase:** Acetonitrile and *Solution A* (7:13)

**Diluent:** Acetonitrile and water (1:1)

**Standard stock solution:** 0.25 mg/mL of USP Anagrelide Hydrochloride RS in acetonitrile. Initially add acetonitrile (about 80% of the volume of the flask) and a small quantity of 2 N hydrochloric acid (about 0.2 mL for every 100 mL of the final volume). Sonicate to dissolve, and dilute with acetonitrile to volume.

**Standard solution:** 0.01 mg/mL of anagrelide free base in *Diluent* from *Standard stock solution*

**Sample solution:** 0.01 mg/mL of anagrelide free base prepared from the contents of NLT 20 Capsules. Add *Diluent* (80% of the volume of the flask), sonicate for

10 min, and stir for 15 min. Further dilute with *Diluent* to volume, centrifuge for 15 min at 4000 rpm, and use the supernatant for analysis.

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 4-μm packing L11

**Column temperature:** 60°

**Flow rate:** 1.0 mL/min

**Injection size:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 3000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>14</sub>H<sub>13</sub>N<sub>5</sub>O<sub>5</sub>S<sub>2</sub>, based on the label claim, in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of anagrelide from the *Sample solution*

$r_S$  = peak response of anagrelide from the *Standard solution*

$C_S$  = concentration of anagrelide in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of anagrelide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION (711)**

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 15 min

**Mobile phase:** Proceed as directed in the *Assay*.

**Standard solution:** Transfer about 30.32 mg of USP Anagrelide Hydrochloride RS, equivalent to 25.00 mg of anagrelide, to a 100-mL volumetric flask. Add about 80 mL of acetonitrile and 3 drops of 2 N hydrochloric acid. Sonicate until dissolved. Dilute with acetonitrile to volume. Dilute this solution with *Medium* to obtain a final concentration of about (L/1000) mg/mL, where L is the Capsule label claim in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 274 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L7

**Sample cooler temperature:** 5°

**Flow rate:** 1.0 mL/min

**Injection size:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 3000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

Calculate the percentage of anagrelide dissolved:

$$\text{Result} = (r_U/r_S) \times W_S(100 - W_C)/(25 \times 100) \times M_{r1}/M_{r2} \times V/L \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$W_S$  = weight of the USP Anagrelide Hydrochloride RS taken (mg)

$W_C$  = water content of the USP Anagrelide Hydrochloride RS (%)



$M_{r1}$  = molecular weight of anagrelide, 256.10  
 $M_{r2}$  = molecular weight of anagrelide hydrochloride, 292.56  
 $V$  = volume of *Medium*, 900 mL  
 $L$  = label claim (mg/Capsule)

Tolerances: NLT 80% (Q) of the labeled amount of anagrelide is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### Organic Impurities

#### • PROCEDURE

**Buffer solution:** 6.8 g/L of monobasic potassium phosphate. Adjust with diluted phosphoric acid to a pH of  $3.50 \pm 0.05$ . Mix well and filter.

**Mobile phase:** Acetonitrile and *Buffer solution* (27:73)  
**Diluent:** Acetonitrile:water (7:13)

**Related compound A stock solution:** 10 µg/mL of USP Anagrelide Related Compound A RS in *Diluent*

**Related compound C stock solution:** 10 µg/mL of USP Anagrelide Related Compound C RS in *Diluent*

**System suitability solution:** 0.2 µg/mL of each USP Anagrelide Related Compound A RS and USP Anagrelide Related Compound C RS and 0.02 mg/mL of USP Anagrelide Hydrochloride RS. Initially dissolve USP Anagrelide Hydrochloride RS in *Diluent* (about 80% of the volume of the flask), sonicate for 10 min, and stir for 15 min. Add appropriate amounts of *Related compound A stock solution* and *Related compound C stock solution*, and dilute with *Diluent* to volume.

**Standard stock solution:** 0.1 mg/mL of anagrelide free base by dissolving USP Anagrelide Hydrochloride RS in acetonitrile (about 80% of the volume of the flask). Add a small quantity of 2 N hydrochloric acid (about 0.2 mL for every 100 mL of the final volume), sonicate to dissolve, and dilute with acetonitrile to volume.

**Standard solution:** 0.10 µg/mL of anagrelide free base in *Diluent* from *Standard stock solution*

**Sample solution:** 0.02 mg/mL of anagrelide free base from NLT 20 Capsules. Initially add *Diluent* to about 80% of the volume of the flask, sonicate for 10 min, stir for about 15 min, and dilute with *Diluent* to volume. Centrifuge (about 4000 rpm) the solution for 15 min, and use the supernatant for analysis.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 4-µm packing L11

**Column temperature:** 45°

**Flow rate:** 1.0 mL/min

**Injection size:** 30 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between anagrelide hydrochloride and anagrelide related compound C, and between anagrelide hydrochloride and anagrelide related compound A, *System suitability solution*

**Column efficiency:** NLT 3000 theoretical plates, *Standard solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0% for the anagrelide peak, *Standard solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of each individual impurity from the *Sample solution*

$r_s$  = peak response of anagrelide from the *Standard solution*

$C_u$  = nominal concentration of anagrelide in the *Sample solution* (mg/mL)

$C_s$  = concentration of anagrelide in the *Standard solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)

**Acceptance criteria:** The individual and total impurities meet the limits in *Impurity Table 1*. [NOTE—Anagrelide related compound A (RRT = 0.86) and anagrelide related compound C (RRT = 1.15) are process related and controlled in the drug substance.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Anagrelide hydrochloride	1.0	—	—
Anagrelide related compound B <sup>a</sup>	0.3	0.34	1.0
Anagrelide trichloro derivative <sup>b</sup>	1.8–2.3	1.0	0.15
Any other individual impurity	—	—	0.2
Total Impurities	—	—	1.5

<sup>a</sup> [2-Amino-5,6-dichloroquinazoline-3(4*H*)-yl]acetic acid.

<sup>b</sup> 6,7,8-Trichloro-3,5-dihydroimidazo[2,1-*b*]quinazolin-2(1*H*)-one.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at controlled room temperature.

#### • USP REFERENCE STANDARDS (11)

USP Anagrelide Hydrochloride RS

USP Anagrelide Related Compound A RS

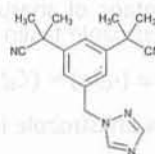
Ethyl 2-(6-amino-2,3-dichlorobenzylamino)acetate.

$C_{11}H_{14}Cl_2N_2O_2$  277.15

USP Anagrelide Related Compound C RS

Ethyl 2-(5,6-dichloro-2-imino-1,2-dihydroquinazolin-3(4*H*)-yl)acetate hydrobromide.

## Anastrozole



$C_{17}H_{19}N_5$  293.37

1,3-Benzenediacetonitrile,  $\alpha,\alpha,\alpha',\alpha'$ -tetramethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-;

$\alpha,\alpha,\alpha',\alpha'$ -Tetramethyl-5-(1*H*-1,2,4-triazol-1-ylmethyl)-*m*-benzenediacetonitrile [120511-73-1].

## DEFINITION

Anastrozole contains NLT 98.0% and NMT 102.0% of anastrozole ( $C_{17}H_{19}N_5$ ), calculated on the anhydrous and solvent-free basis.

## IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.



**ASSAY****• PROCEDURE**

**Solution A:** Acetonitrile, methanol, trifluoroacetic acid, and water (100: 300: 0.5: 600)

**Solution B:** Acetonitrile, methanol, trifluoroacetic acid, and water (150: 450: 0.5: 400)

**Mobile phase:** See Table 1.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
40	0	100
41	100	0
56	100	0

[NOTE—These gradient elution times are established on an HPLC system with a dwell time of approximately 0 min. The gradient elution times in the table can be adjusted by subtracting the dwell time to achieve the separation described.]

**Standard solution:** 0.5 mg/mL of USP Anastrozole RS prepared as follows. Transfer USP Anastrozole RS into a suitable volumetric flask. Dissolve in acetonitrile, using 40% of the final volume, and then dilute with *Solution A* to volume.

**Sample solution:** 0.5 mg/mL of Anastrozole prepared as follows. Transfer 25 mg of Anastrozole to a 50-mL volumetric flask, add 20 mL of acetonitrile to dissolve. Dilute with *Solution A* to volume.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 3.2-mm × 10-cm; 5-μm packing L42

**Flow rate:** 0.75 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** Between 0.9 and 1.4

**Relative standard deviation:** NMT 0.73%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of anastrozole (C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>) in the portion of Anastrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of anastrozole from the *Sample solution*

$r_S$  = peak area of anastrozole from the *Standard solution*

$C_S$  = concentration of USP Anastrozole RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Anastrozole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis

**IMPURITIES**

- RESIDUE ON IGNITION** <281>: NMT 0.1%

**Delete the following:**

- HEAVY METALS, Method II** <231>: NMT 10 ppm (Official 1-Jan-2018)

**• ORGANIC IMPURITIES**

**Solution A, Solution B, and Chromatographic system:** Proceed as directed in the Assay.

**Standard stock solution:** 0.2 mg/mL of USP Anastrozole RS prepared as follows. Dissolve in acetonitrile,

using 40% of the final volume, and then dilute with *Solution A* to volume.

**Standard solution:** 0.02 mg/mL of USP Anastrozole RS in *Solution A* from the *Standard stock solution*

**Sample solution:** 2 mg/mL of Anastrozole prepared as follows. Transfer 50 mg of Anastrozole to a 25-mL volumetric flask. Add 10 mL of acetonitrile. Dissolve in and dilute with *Solution A* to volume.

**Blank solution:** Transfer 10 mL of acetonitrile into a 25-mL volumetric flask, and dilute with *Solution A* to volume.

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** Between 0.9 and 1.4

**Relative standard deviation:** NMT 5%

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank solution*. [NOTE—Adjust the peak areas for any interference from the *Blank solution*.]

Calculate the percentage of each individual impurity in the portion of Anastrozole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of each individual impurity from the *Sample solution*

$r_S$  = peak area of anastrozole from the *Standard solution*

$C_S$  = concentration of USP Anastrozole RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Anastrozole in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 2. Disregard any impurity of less than 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desmethyl anastrozole <sup>a</sup>	0.6	0.2
Anastrozole	1.0	—
Anastrozole dimer <sup>b</sup>	2.0	0.2
5-Bromomethyl anastrozole <sup>c</sup>	4.3	0.1
5-Dibromomethyl anastrozole <sup>d</sup>	5.4	0.1
Individual unspecified impurity	—	0.1
Total impurities	—	0.5

<sup>a</sup> 2-(3-(1-Cyanoethyl)-5-(1H-1,2,4-triazol-1-ylmethyl)phenyl)-2-methylpropionitrile.

<sup>b</sup> 2,3-Bis(3-(1-cyano-1-methylethyl)-5-(1H-1,2,4-triazol-1-ylmethyl)phenyl)-2-methylpropionitrile.

<sup>c</sup> 2,2'-(5-(Bromomethyl)-1,3-phenylene)bis(2-methylpropionitrile).

<sup>d</sup> 2,2'-(5-(Dibromomethyl)-1,3-phenylene)bis(2-methylpropionitrile).

**SPECIFIC TESTS**

- WATER DETERMINATION, Method Ic** <921>: NMT 0.3%

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

- USP REFERENCE STANDARDS (11)**  
USP Anastrozole RS

**Anastrozole Tablets****DEFINITION**

Anastrozole Tablets contain NLT 90% and NMT 110% of the labeled amount of anastrozole (C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>).



**IDENTIFICATION**• **A. INFRARED ABSORPTION (17K)**

**Sample:** Transfer the finely ground Tablet powder containing 8 mg of anastrozole into a suitable container. Add 10 mL of diethyl ether and sonicate for 5 min. Aspirate the supernatant and pass the slurry through a nylon filter of 0.45- $\mu$ m pore size into another suitable container containing 400 mg of spectroscopic grade potassium bromide. Evaporate the mixture to dryness under nitrogen. Further dry it under vacuum at 50° for 1 h.

**Acceptance criteria:** Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Acetonitrile and water (40:60)

**Diluent:** Acetonitrile and water (50:50)

**Standard solution:** 40  $\mu$ g/mL of USP Anastrozole RS in *Diluent*. Sonication may be used to aid dissolution.

**Sample solution:** Nominally equivalent to 40  $\mu$ g/mL of anastrozole in *Diluent*, prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask. Add 40% of the flask volume of water, and shake on a rotary shaker for 10 min to disintegrate the Tablets. Add 40% of the flask volume of acetonitrile, and sonicate for 15 min with intermittent shaking, maintaining the sonicator temperature at 25°. Dilute with *Diluent* to volume. Centrifuge a portion of the solution at 3500 rpm for 10 min, and use the clear solution for analysis.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anastrozole ( $C_{17}H_{19}N_5$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Anastrozole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of anastrozole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90%–110%

**PERFORMANCE TESTS**• **DISSOLUTION (711)****Test 1**

**Medium:** Water; 900 mL, deaerated

**Apparatus 2:** 50 rpm

**Time:** 15 min

**Mobile phase:** Acetonitrile and water (40:60)

**Diluent:** Acetonitrile and water (50:50)

**Standard stock solution:** 0.2 mg/mL of USP Anastrozole RS in *Diluent*

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (L/1000) mg/mL, where L is the label claim in mg/Tablet.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size. Discard the first few mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 50  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anastrozole ( $C_{17}H_{19}N_5$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of anastrozole ( $C_{17}H_{19}N_5$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** Water; 1000 mL, deaerated

**Apparatus 2:** 50 rpm

**Time:** 15 min

**Mobile phase:** Acetonitrile, trifluoroacetic acid, and water (300:1:700)

**Standard stock solution:** 0.2 mg/mL of USP Anastrozole RS prepared as follows. Transfer USP Anastrozole RS into a suitable volumetric flask and add acetonitrile equivalent to 8% of the final volume. Sonicate to dissolve and dilute with water to volume.

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of (L/1000) mg/mL, where L is the label claim in mg/Tablet.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size. Discard the first few mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 3.2-mm  $\times$  10-cm; 5- $\mu$ m packing L42

**Flow rate:** 0.75 mL/min

**Injection volume:** 100  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** 0.9–1.4

**Relative standard deviation:** NMT 1.5%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anastrozole ( $C_{17}H_{19}N_5$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

L = label claim (mg/Tablet)

V = volume of *Medium*, 1000 mL



**Tolerances:** NLT 80% (Q) of the labeled amount of anastrozole (C<sub>17</sub>H<sub>19</sub>N<sub>3</sub>) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Solution A:** Methanol, acetonitrile, trifluoroacetic acid, and water (200: 100: 0.7: 700)

**Solution B:** Methanol, acetonitrile, trifluoroacetic acid, and water (500: 250: 0.7: 250)

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25	100	0
25.1	0	100
30	0	100
31	100	0
40	100	0

**Diluent:** Acetonitrile, trifluoroacetic acid, and water (200: 0.8: 800)

**System suitability stock solution:** 0.5 mg/mL of USP Anastrozole RS and 0.3 mg/mL of ethyl 4-hydroxybenzoate in *Diluent* prepared as follows. Transfer USP Anastrozole RS and ethyl 4-hydroxybenzoate into a suitable volumetric flask and add *Diluent* equivalent to 50% of the final volume. Sonicate to dissolve and dilute with *Diluent* to volume.

**System suitability solution:** 10 µg/mL of USP Anastrozole RS and 6 µg/mL of ethyl 4-hydroxybenzoate in *Diluent*, from *System suitability stock solution*

**Standard stock solution:** 0.5 mg/mL of USP Anastrozole RS in *Diluent* prepared as follows. Transfer USP Anastrozole RS into a suitable volumetric flask and add *Diluent* equivalent to 50% of the final volume. Sonicate to dissolve and dilute with *Diluent* to volume.

**Standard solution:** 10 µg/mL of USP Anastrozole RS in *Diluent*, from *Standard stock solution*

**Sample solution:** Nominally equivalent to 1.0 mg/mL of anastrozole from NLT 25 finely powdered Tablets, prepared as follows. Transfer a weighed quantity of powdered Tablets, equivalent to 10 mg of anastrozole, to a suitable container and add 10.0 mL of *Diluent*. Sonicate for 30 min and allow to cool to room temperature. Pass through a suitable filter of 0.45-µm pore size, and discard the first few mL of the filtrate. If the filtrate is not clear, pass again through a suitable filter of 0.2-µm pore size, and discard the first few mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 3.2-mm × 10-cm; 5-µm packing L42

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 µL

**Analysis time:** 25 min

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for ethyl 4-hydroxybenzoate and anastrozole are 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** Greater than 4 between ethyl 4-hydroxybenzoate and anastrozole peaks

**Tailing factor:** 0.9–1.3, for the anastrozole peak

**Relative standard deviation:** NMT 5%, for the anastrozole peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of anastrozole from the *Standard solution*

$C_S$  = concentration of USP Anastrozole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of anastrozole in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 2. Disregard any impurity peak less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Anastrozole diamide <sup>a</sup>	0.11	0.5
Anastrozole monoacid monoamide <sup>b</sup>	0.26	0.5
Anastrozole monoamide mononitrile <sup>c</sup>	0.30	0.5
Desmethyl anastrozole <sup>d</sup>	0.51	—
Anastrozole diacid <sup>e</sup>	0.71	0.5
Anastrozole monoacid mononitrile <sup>f</sup>	0.87	0.5
Anastrozole	1.00	—
Any individual unspecified impurity	—	0.5
Total impurities	—	1.0

<sup>a</sup> 2,2'-(5-[(1H-1,2,4-Triazol-1-yl)methyl]-1,3-phenylene)bis(2-methylpropanamide).

<sup>b</sup> 2-{3-[(1H-1,2,4-Triazol-1-yl)methyl]-5-(1-amino-2-methyl-1-oxopropan-2-yl)phenyl}-2-methylpropanoic acid.

<sup>c</sup> 2-{3-[(1H-1,2,4-Triazol-1-yl)methyl]-5-(2-cyanopropan-2-yl)phenyl}-2-methylpropanamide.

<sup>d</sup> 2-(3-(1-Cyanoethyl)-5-(1H-1,2,4-triazol-1-yl)methyl)phenyl)-2-methylpropanenitrile. This process impurity is controlled in the drug substance monograph. It is included in the table for identification only, and it is not to be reported in the *Total impurities*.

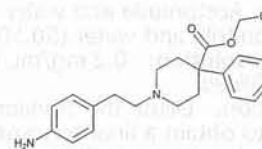
<sup>e</sup> 2,2'-(5-[(1H-1,2,4-Triazol-1-yl)methyl]-1,3-phenylene)bis(2-methylpropanoic acid).

<sup>f</sup> 2-{3-[(1H-1,2,4-Triazol-1-yl)methyl]-5-(2-cyanopropan-2-yl)phenyl}-2-methylpropanoic acid.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**  
USP Anastrozole RS

## Anileridine



C<sub>22</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>

352.47



4-Piperidinecarboxylic acid, 1-[2-(4-aminophenyl)ethyl]-4-phenyl-, ethyl ester;  
Ethyl 1-(*p*-aminophenethyl)-4-phenylisonipecotatate  
[144-14-9].

**DEFINITION**

Anileridine contains NLT 98.5% and NMT 101.0% of anileridine ( $C_{22}H_{28}N_2O_2$ ), calculated on the anhydrous basis.

**IDENTIFICATION**• **A.**

**Buffer solution:** Dissolve 5.68 g of anhydrous dibasic sodium phosphate and 3.63 g of monobasic potassium phosphate in water to make 1000 mL; the pH is 7.0  $\pm$  0.05.

**Sample stock solution:** Dissolve 40 mg of Anileridine in 2.3 mL of 0.1 N hydrochloric acid in a 100-mL volumetric flask, and dilute with water to volume.

**Sample solution A:** *Sample stock solution, Buffer solution, and water (4:25:71)*

**Sample solution B:** *Sample stock solution, Buffer solution, and water (20:25:55)*

**Acceptance criteria:** The UV absorption spectrum of *Sample solution A* exhibits a maximum at 234  $\pm$  1 nm; and the UV absorption spectrum of *Sample solution B* exhibits a maximum at 285  $\pm$  2 nm. The ratio  $5A_{234}/A_{285}$  is 8.8.

• **B.**

**Sample solution:** 0.2 mg/mL of Anileridine in 0.1 N hydrochloric acid

**Analysis:** To 5 mL of the *Sample solution* add 2 mL of a solution of *p*-dimethylaminobenzaldehyde in alcohol (1 in 100).

**Acceptance criteria:** A yellow color develops immediately.

**ASSAY**• **PROCEDURE**

**Sample:** 350 mg of Anileridine

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with *Titrant* to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 17.62 mg of anileridine ( $C_{22}H_{28}N_2O_2$ ).

**Acceptance criteria:** 98.5%–101.0% on the anhydrous basis

**IMPURITIES**

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **CHLORIDE AND SULFATE, Chloride** (221)

**Sample:** 180 mg

**Analysis:** Dissolve the *Sample* in a mixture of 1 mL of nitric acid and 40 mL of water.

**Acceptance criteria:** NMT 400 ppm; the solution shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid.

**SPECIFIC TESTS**

• **WATER DETERMINATION, Method I** (921): NMT 1.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.

**Anileridine Injection****DEFINITION**

Anileridine Injection is a sterile solution of Anileridine in Water for Injection, prepared with the aid of Phosphoric Acid. It contains NLT 90.0% and NMT 115.0% of the labeled amount of anileridine ( $C_{22}H_{28}N_2O_2$ ), as the phosphate.

**IDENTIFICATION**• **A.**

**Sample solution:** 0.25 mg/mL of anileridine from Injection diluted with water

**Analysis:** To 5 mL of the *Sample solution* add 2 mL of a 10-mg/mL solution of *p*-dimethylaminobenzaldehyde in alcohol.

**Acceptance criteria:** A yellow color develops immediately.

• **B.** A volume of Injection, diluted with water to a concentration of 0.025 mg/mL of anileridine, exhibits absorbance maxima at 234  $\pm$  1 and 285  $\pm$  2 nm.

**ASSAY**• **PROCEDURE**

**Standard solution:** Prepare 250  $\mu$ g/mL of USP Anileridine Hydrochloride RS in 0.1 N hydrochloric acid on the day of the assay. Each mg of anileridine hydrochloride is equivalent to 0.8286 mg of anileridine.

**Sample solution:** Nominally equivalent to 200  $\mu$ g/mL of anileridine, from a volume of Injection diluted with 0.1 N hydrochloric acid

**Blank:** 0.1 N hydrochloric acid

**Instrumental conditions**

**Analytical wavelength:** 560 nm

**Cell:** 1 cm

**Analysis**

**Samples:** *Standard solution, Sample solution, and Blank*  
Transfer 5.0 mL each of the *Standard solution, Sample solution, and Blank* to separate 200-mL volumetric flasks. To each flask add 25 mL of water, 5 mL of 1 N hydrochloric acid, and 5 mL of 1-mg/mL sodium nitrite solution. Allow to stand for 2 min, then add to each flask 5 mL of 5-mg/mL ammonium sulfamate solution. Allow to stand for 3 min, then add 5 mL of 1-mg/mL *N*-(1-naphthyl)ethylenediamine dihydrochloride solution. Allow to stand for 1 h, and dilute with water to volume. Use the reagent blank to set the instrument.

Calculate the percentage of anileridine ( $C_{22}H_{28}N_2O_2$ ) in the volume of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Anileridine Hydrochloride RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of anileridine in the *Sample solution* ( $\mu$ g/mL)

$M_{r1}$  = molecular weight of anileridine, 352.48

$M_{r2}$  = molecular weight of anileridine hydrochloride, 425.40

**Acceptance criteria:** 90.0%–115.0%

**SPECIFIC TESTS**

• **pH** (791): 4.5–5.0

• **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 7.2 USP Endotoxin Units/mg of anileridine.

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.
- **USP REFERENCE STANDARDS (11)**  
USP Anileridine Hydrochloride RS  
USP Endotoxin RS

**Anileridine Hydrochloride**

$C_{22}H_{28}N_2O_2 \cdot 2HCl$  425.39  
4-Piperidinecarboxylic acid, 1-[2-(4-aminophenyl)ethyl]-4-phenyl-, ethyl ester, dihydrochloride;  
Ethyl 1-(*p*-aminophenethyl)-4-phenylisopropylate dihydrochloride [126-12-5].

**DEFINITION**

Anileridine Hydrochloride contains NLT 96.0% and NMT 102.0% of anileridine hydrochloride ( $C_{22}H_{28}N_2O_2 \cdot 2HCl$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**

• **B.**

**Buffer solution:** 5.68 g/L of anhydrous dibasic sodium phosphate and 3.63 g/L of monobasic potassium phosphate in water: the pH, determined potentiometrically, is  $7.0 \pm 0.05$ .

**Sample stock solution:** 0.5 mg/mL of anileridine hydrochloride in water

**Sample solution A:** *Sample stock solution*, pH 7.0 *buffer solution*, and water (4:25:71)

**Sample solution B:** *Sample stock solution*, pH 7.0 *buffer solution*, and water (20:25:55)

**Acceptance criteria:** The UV absorption spectrum of *Sample solution A* exhibits a maximum at  $234 \pm 1$  nm, and the UV absorption spectrum of *Sample solution B* exhibits a maximum at  $285 \pm 2$  nm.

• **C.**

**Sample solution:** 0.2 mg/mL of Anileridine Hydrochloride

**Analysis:** To 5 mL of the *Sample solution* add 2 mL of a solution of 10 mg/mL of *p*-dimethylaminobenzaldehyde in alcohol.

**Acceptance criteria:** A yellow color develops immediately.

- **D. IDENTIFICATION TESTS—GENERAL, Chloride (191)**

**Sample:** 10-mg/mL solution of Anileridine Hydrochloride

**Acceptance criteria:** Meets the requirements

**ASSAY**• **PROCEDURE**

**Sample:** 200 mg of Anileridine Hydrochloride  
**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 10 mL of glacial acetic acid by heating it on a steam bath. Cool immediately in a cold water bath, add 5 mL of mercuric acetate TS, 20 mL of acetone, and 0.5 mL of indicator solution (70 mg of  $\alpha$ -naphtholbenzein, 10 mg of crystal violet, and 40 mg of quinaldine red in 100 mL of glacial acetic acid). Titrate with *Titrant* to a gray-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 21.27 mg of anileridine hydrochloride ( $C_{22}H_{28}N_2O_2 \cdot 2HCl$ ).

**Acceptance criteria:** 96.0%–102.0% on the dried basis

**OTHER COMPONENTS**• **CONTENT OF CHLORIDE**

**Sample:** 200 mg

**Analysis:** Dissolve the *Sample* in 50 mL of water in a glass-stoppered flask. Add 25.0 mL of 0.1 N silver nitrate VS, then add 5 mL of 2 N nitric acid and 5 mL of nitrobenzene, shake vigorously, and add 2 mL of ferric ammonium sulfate TS. Titrate the excess silver nitrate with 0.1 N ammonium thiocyanate VS. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl.

**Acceptance criteria:** 16.0%–17.2%

**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 0.1%

**SPECIFIC TESTS**• **PH (791)**

**Sample solution:** 50 mg/mL

**Acceptance criteria:** 2.5–3.0

• **LOSS ON DRYING (731)**

**Analysis:** Dry a sample at a pressure below 5 mm of mercury at 100° for 2 h.

**Acceptance criteria:** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP Anileridine Hydrochloride RS

**Anileridine Hydrochloride Tablets****DEFINITION**

Anileridine Hydrochloride Tablets contain an amount of anileridine hydrochloride ( $C_{22}H_{28}N_2O_2 \cdot 2HCl$ ) equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of anileridine ( $C_{22}H_{28}N_2O_2$ ).

**IDENTIFICATION**• **A.**

**Sample solution:** Transfer an equivalent to 50 mg of anileridine, from finely powdered Tablets, to a 250-mL volumetric flask. Add 100 mL of water, and heat on a steam bath. Cool, dilute to volume, and filter.

**Analysis:** To 5 mL of the *Sample solution* add 2 mL of a 10-mg/mL solution of *p*-dimethylaminobenzaldehyde in alcohol.

**Acceptance criteria:** A yellow color develops immediately.

• **B.**

**Buffer solution:** 5.68 g/L of anhydrous dibasic sodium phosphate and 3.63 g/L of monobasic potassium phosphate in water: the pH, determined potentiometrically, is  $7.0 \pm 0.05$ .

**Sample stock solution:** Transfer an equivalent to 50 mg of anileridine, from finely powdered Tablets, to a 100-mL volumetric flask. Add 30 mL of water, and heat on a steam bath. Cool, dilute with water to volume, and filter.

**Sample solution A:** *Sample stock solution*, *Buffer solution*, and water (4:25:71)

**Sample solution B:** *Sample stock solution*, *Buffer solution*, and water (20:25:55)

**Acceptance criteria:** The UV absorption spectrum of *Sample solution A* exhibits a maximum at  $234 \pm 1$  nm, and the UV absorption spectrum of *Sample solution B* exhibits a maximum at  $285 \pm 2$  nm.



**ASSAY****• PROCEDURE**

**Standard solution:** 250 µg/mL of USP Anileridine Hydrochloride RS in 0.1 N hydrochloric acid. (Each mg of anileridine hydrochloride is equivalent to 0.8286 mg of anileridine.) Prepare on the day of the assay.

**Sample solution:** Transfer an equivalent to 50 mg of anileridine, from finely powdered Tablets (NLT 20), to a 250-mL volumetric flask. Add 25 mL of 1 N hydrochloric acid and 100 mL of water, and heat on a water bath. Cool, and dilute with water to volume. Filter the solution, discarding the first 25 mL of the filtrate.

**Blank:** 0.1 N hydrochloric acid

**Instrumental conditions**

**Analytical wavelength:** 560 nm

**Cell:** 1 cm

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank* Transfer 5.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to separate 200-mL volumetric flasks. To each flask add 25 mL of water, 5 mL of 1 N hydrochloric acid, and 5 mL of 1-mg/mL sodium nitrite solution. Allow to stand for 2 min, then add to each flask 5 mL of 5-mg/mL ammonium sulfamate solution. Allow to stand for 3 min, then add 5 mL of 1-mg/mL *N*-(1-naphthyl)ethylenediamine dihydrochloride solution. Allow to stand for 1 h, and dilute with water to volume. Use the reagent blank to set the instrument.

Calculate the percentage of anileridine ( $C_{22}H_{28}N_2O_2$ ) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$	= absorbance of the solution from the <i>Sample solution</i>
$A_S$	= absorbance of the solution from the <i>Standard solution</i>
$C_S$	= concentration of USP Anileridine Hydrochloride RS in the <i>Standard solution</i> (µg/mL)
$C_U$	= nominal concentration of anileridine in the <i>Sample solution</i> (µg/mL)
$M_{r1}$	= molecular weight of anileridine, 352.48
$M_{r2}$	= molecular weight of anileridine hydrochloride, 425.40

**Acceptance criteria:** 95.0%–105.0%

**PERFORMANCE TESTS****• DISSOLUTION (711)**

**Medium:** 0.01 N hydrochloric acid; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Standard solution:** USP Anileridine Hydrochloride RS of a known concentration, in *Medium*

**Sample solution:** Filtered portion of the solution under test

**Analysis**

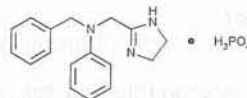
**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of anileridine ( $C_{22}H_{28}N_2O_2$ ) dissolved, using the *Analysis* set forth in the *Assay* and in comparison to the *Standard solution*.

**Tolerances:** NLT 65% (Q) of the labeled amount of anileridine ( $C_{22}H_{28}N_2O_2$ ) is dissolved.

**• UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.**• USP REFERENCE STANDARDS (11)**

USP Anileridine Hydrochloride RS

**Antazoline Phosphate**

$C_{17}H_{19}N_3 \cdot H_3PO_4$  363.35

1*H*-Imidazole-2-methanamine, 4,5-dihydro-*N*-phenyl-*N*-(phenylmethyl)-, phosphate (1:1);

2-[(*N*-Benzylanilino)methyl]-2-imidazoline phosphate (1:1) [154-68-7].

**DEFINITION**

Antazoline Phosphate contains NLT 98.0% and NMT 102.0% of antazoline phosphate ( $C_{17}H_{19}N_3 \cdot H_3PO_4$ ), calculated on the dried basis.

**IDENTIFICATION****• A. INFRARED ABSORPTION (197M)****• B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY****• PROCEDURE**

**Solution A:** Dilute 1.0 mL of formic acid with water to 1000 mL.

**Mobile phase:** Acetonitrile and *Solution A* (17:83)

**Diluent:** Acetonitrile and water (17:83)

**Standard solution:** 0.2 mg/mL of USP Antazoline Phosphate RS in *Diluent*

**System suitability solution:** 1 µg/mL of USP Antazoline Related Compound A RS in the *Standard solution*

**Sample solution:** 0.2 mg/mL of Antazoline Phosphate in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 2.1-mm × 10-cm; 1.7-µm packing L11

**Temperatures**

**Column:** 35°

**Autosampler:** 4°

**Flow rate:** 0.5 mL/min

**Injection volume:** 5 µL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times of antazoline and antazoline related compound A are 1.0 and 1.1, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between antazoline and antazoline related compound A, *System suitability solution*

**Tailing factor:** NMT 1.5 for antazoline, *Standard solution*

**Relative standard deviation:** NMT 0.73% for antazoline, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of antazoline phosphate ( $C_{17}H_{19}N_3 \cdot H_3PO_4$ ) in the portion of Antazoline Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Antazoline Phosphate RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Antazoline Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

## IMPURITIES

### • ORGANIC IMPURITIES

**Solution A:** Dilute 1.0 mL of formic acid with water to 1000 mL.

**Mobile phase:** Acetonitrile and *Solution A* (17:83)

**Diluent:** Acetonitrile and water (17:83)

**Standard solution A:** 5 µg/mL of USP Antazoline Related Compound A RS and 1.0 mg/mL of USP Antazoline Phosphate RS in *Diluent*

**Standard solution B:** 1 µg/mL of USP Antazoline Phosphate RS in *Diluent*

**Sample solution:** 1.0 mg/mL of Antazoline Phosphate in *Diluent*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 2.1-mm × 10-cm; 1.7-µm packing L11

**Temperatures**

**Column:** 35°

**Autosampler:** 4°

**Flow rate:** 0.5 mL/min

**Injection volume:** 5 µL

**Run time:** NLT 4 times the retention time of antazoline

### System suitability

**Samples:** *Standard solution A* and *Standard solution B*

[NOTE—The relative retention times of antazoline and antazoline related compound A are 1.0 and 1.1, respectively.]

### Suitability requirements

**Resolution:** NLT 2.0 between antazoline and antazoline related compound A peaks, *Standard solution A*

**Relative standard deviation:** NMT 5.0% for antazoline, *Standard solution B*

### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of antazoline related compound A in the portion of Antazoline Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of antazoline related compound A from the *Sample solution*  
 $r_S$  = peak response of antazoline related compound A from *Standard solution A*  
 $C_S$  = concentration of USP Antazoline Related Compound A RS in *Standard solution A* (mg/mL)  
 $C_U$  = concentration of Antazoline Phosphate in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Antazoline Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of any impurity from the *Sample solution*  
 $r_S$  = peak response of antazoline phosphate from *Standard solution B*  
 $C_S$  = concentration of USP Antazoline Phosphate RS in *Standard solution B* (mg/mL)  
 $C_U$  = concentration of Antazoline Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Antazoline phosphate	1.0	—
Antazoline related compound A <sup>a</sup>	1.1	0.5
Any individual unspecified impurity	—	0.5
Total impurities	—	1.0

<sup>a</sup> N-(2-Aminoethyl)-2-[benzyl(phenyl)amino]acetamide.

## SPECIFIC TESTS

### • PH (791)

**Sample:** 20 mg/mL

**Acceptance criteria:** 4.0–5.0

### • LOSS ON DRYING (731)

**Analysis:** Dry at 105° for 4 h.

**Acceptance criteria:** NMT 0.5%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

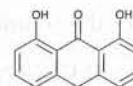
USP Antazoline Phosphate RS

USP Antazoline Related Compound A RS

N-(2-Aminoethyl)-2-[benzyl(phenyl)amino]acetamide.

C<sub>17</sub>H<sub>21</sub>N<sub>3</sub>O 283.38

## Anthralin



C<sub>14</sub>H<sub>10</sub>O<sub>3</sub>

226.23

9(10H)-Anthracenone, 1,8-dihydroxy-;  
1,8-Dihydroxy-9-anthrone [1143-38-0].

## DEFINITION

Anthralin contains NLT 97.0% and NMT 102.0% of anthralin (C<sub>14</sub>H<sub>10</sub>O<sub>3</sub>), calculated on the dried basis.

## IDENTIFICATION

• **A. INFRARED ABSORPTION (197K)**

• **B. ULTRAVIOLET ABSORPTION (197U)**

**Sample solution:** 10 µg/mL in chloroform

**Acceptance criteria:** Meets the requirements

## ASSAY

### • PROCEDURE

[NOTE—Use low-actinic glassware.]

**Mobile phase:** *n*-Hexane, dichloromethane, and glacial acetic acid (82:12:6)

**Internal standard solution:** 0.5 mg/mL of *o*-nitroaniline in *n*-hexane prepared as follows. First dissolve *o*-nitroaniline in a small quantity of dichloromethane, and then dilute with *n*-hexane.

**System suitability stock solution:** 0.1 mg/mL of USP Anthralin RS and 0.2 mg/mL of danthron in dichloromethane

**System suitability solution:** Transfer 5 mL of the *System suitability stock solution* into a 25-mL volumetric flask, add 5 mL of *n*-hexane, and dilute with *Mobile phase* to volume.

**Solvent blank solution:** *Mobile phase*, *n*-hexane, and dichloromethane (3:1:1)



**Standard stock solution:** 0.25 mg/mL of USP Anthralin RS in dichloromethane

**Standard solution:** Transfer 5 mL each of *Standard stock solution* and *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample stock solution:** 0.25 mg/mL of Anthralin in dichloromethane

**Sample solution:** Transfer 5 mL each of *Sample stock solution* and *Internal standard solution* into a 25-mL volumetric flask, dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 354 nm

**Column:** 4.6-mm × 25-cm; packing L3

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Samples:** *System suitability solution*, *Solvent blank solution*, and *Standard solution*

[NOTE—The relative retention times for anthralin, danthron, dianthrone, and o-nitroaniline are 1.0, 1.2, 1.7, and 2.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.3 between anthralin and danthron, *System suitability solution*

**Tailing factor:** NMT 1.5, *System suitability solution*

**Relative standard deviation:** NMT 2.0% of the ratio of the peak responses, *Standard solution*

**Interference:** No discernible signal is observed at the retention time of anthralin, *Solvent blank solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of anthralin ( $C_{14}H_{10}O_3$ ) in the portion of Anthralin taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of anthralin to o-nitroaniline from the *Sample solution*

$R_S$  = peak response ratio of anthralin to o-nitroaniline from the *Standard solution*

$C_S$  = concentration of USP Anthralin RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Anthralin in the *Sample solution* (µg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

#### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **CHLORIDE AND SULFATE, Chloride** (221)

**Sample:** 1 g

**Analysis:** To 15 mL of water add the *Sample*, mix, and filter. Acidify 5 mL of the filtrate with nitric acid, and add a few drops of silver nitrate TS.

**Acceptance criteria:** No more opalescence is produced immediately than is present in a 5-mL portion of the filtrate to which nothing has been added.

• **CHLORIDE AND SULFATE, Sulfate** (221)

**Sample:** 5 mL of the untreated filtrate obtained in the test for *Chloride*

**Analysis:** To the *Sample* add 3 drops of 3 N hydrochloric acid and 5 drops of barium chloride TS.

**Acceptance criteria:** No more turbidity is produced than is present in a 5-mL portion of the filtrate to which nothing has been added.

#### SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE, Class I** (741): 178°–181°

• **LOSS ON DRYING** (731)

**Analysis:** Dry a sample over silica gel for 4 h.

**Acceptance criteria:** NMT 0.5%

• **ACIDITY OR ALKALINITY**

**Analysis:** Suspend a sample in water, and filter.

**Acceptance criteria:** The filtrate is neutral to litmus.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers in a cool place. Protect from light.

• **USP REFERENCE STANDARDS** (11)

USP Anthralin RS

## Anthralin Cream

#### DEFINITION

Anthralin Cream is Anthralin in an aqueous (oil-in-water) or oily (water-in-oil) cream vehicle. Cream labeled to contain more than 0.1% of anthralin contains NLT 90.0% and NMT 115.0% of the labeled amount of anthralin ( $C_{14}H_{10}O_3$ ), and Cream labeled to contain 0.1% or less of anthralin contains NLT 90.0% and NMT 130.0% of the labeled amount of anthralin ( $C_{14}H_{10}O_3$ ).

#### ASSAY

##### • PROCEDURE

[NOTE—Use low-actinic glassware.]

**Mobile phase:** *n*-Hexane, dichloromethane, and glacial acetic acid (82:12:6)

**Internal standard solution:** 0.5 mg/mL of o-nitroaniline in *n*-hexane prepared as follows. First dissolve o-nitroaniline in a small quantity of dichloromethane, and then dilute with *n*-hexane.

**System suitability stock solution:** 0.1 mg/mL of USP Anthralin RS and 0.2 mg/mL of danthron in dichloromethane

**System suitability solution:** Transfer 5 mL of the *System suitability stock solution* into a 25-mL volumetric flask, add 5 mL of *n*-hexane, and dilute with *Mobile phase* to volume.

**Solvent blank solution:** *Mobile phase*, *n*-hexane, and dichloromethane (3:1:1)

**Standard stock solution:** 0.25 mg/mL of USP Anthralin RS in dichloromethane

**Standard solution:** Transfer 2 mL each of *Standard stock solution* and *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample stock solution:** Weigh 5 g of Cream into a 100-mL beaker. Add 20 mL of dichloromethane and 10 mL of glacial acetic acid, and stir to disperse the Cream. Transfer the contents of the beaker to a filter paper (Whatman No. 4, or equivalent) with the aid of dichloromethane, and filter into a 100-mL volumetric flask. Thoroughly wash the precipitate with dichloromethane, and allow the washings to drain into the flask. Dilute with dichloromethane to volume.

**Sample solution:** Transfer a volume of *Sample stock solution* equivalent to 0.5 mg of anthralin and 2 mL of *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 354 nm

**Column:** 4.6-mm × 25-cm; packing L3

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Samples:** *System suitability solution*, *Solvent blank solution*, and *Standard solution*



[NOTE—The relative retention times for anthralin, danthron, dianthrone, and *o*-nitroaniline are 1.0, 1.2, 1.7, and 2.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.3 between anthralin and danthron, *System suitability solution*

**Tailing factor:** NMT 1.5, *System suitability solution*

**Relative standard deviation:** NMT 2.0% of the ratio of the peak responses, *Standard solution*

**Interference:** No discernible signal is observed at the retention time of anthralin, *Solvent blank solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of anthralin ( $C_{14}H_{10}O_3$ ) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of anthralin to *o*-nitroaniline from the *Sample solution*

$R_S$  = peak response ratio of anthralin to *o*-nitroaniline peak from the *Standard solution*

$C_S$  = concentration of USP Anthralin RS in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of anthralin in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–115.0% for Cream labeled to contain more than 0.1% of anthralin;  
90.0%–130.0% for Cream labeled to contain 0.1% or less of anthralin

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, in a cool place. Protect from light.
- **LABELING:** Label it to indicate whether the cream vehicle is aqueous or oily.
- **USP REFERENCE STANDARDS** <11>  
USP Anthralin RS

### Anthralin Ointment

#### DEFINITION

Anthralin Ointment is Anthralin in a petrolatum or other oleaginous vehicle. Ointment labeled to contain more than 0.1% of anthralin contains NLT 90.0% and NMT 115.0% of the labeled amount of anthralin ( $C_{14}H_{10}O_3$ ), and Ointment labeled to contain 0.1% or less of anthralin contains NLT 90.0% and NMT 130.0% of the labeled amount of anthralin ( $C_{14}H_{10}O_3$ ).

#### ASSAY

##### • PROCEDURE

[NOTE—Use low-actinic glassware.]

**Mobile phase:** *n*-Hexane, dichloromethane, and glacial acetic acid (82:12:6)

**Internal standard solution:** 0.5 mg/mL of *o*-nitroaniline in *n*-hexane prepared as follows. First dissolve *o*-nitroaniline in a small quantity of dichloromethane, and then dilute with *n*-hexane.

**System suitability stock solution:** 0.1 mg/mL of USP Anthralin RS and 0.2 mg/mL of danthron in dichloromethane

**System suitability solution:** Transfer 5 mL of the *System suitability stock solution* into a 25-mL volumetric flask, add 5 mL of *n*-hexane, and dilute with *Mobile phase* to volume.

**Solvent blank solution:** *Mobile phase*, *n*-hexane, and dichloromethane (3:1:1)

**Standard stock solution:** 0.25 mg/mL of USP Anthralin RS in dichloromethane

**Standard solution:** Transfer 2 mL each of *Standard stock solution* and *Internal standard solution* into a

25-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample stock solution:** Weigh 5 g of Ointment into a 100-mL beaker. Add 20 mL of dichloromethane and 10 mL of glacial acetic acid, and stir to disperse the Ointment. Transfer the contents of the beaker to a filter paper (Whatman No. 4, or equivalent) with the aid of dichloromethane, and filter into a 100-mL volumetric flask. Thoroughly wash the precipitate with dichloromethane, and allow the washings to drain into the flask. Dilute with dichloromethane to volume.

**Sample solution:** Transfer a volume of *Sample stock solution* equivalent to 0.5 mg of anthralin and 2 mL of *Internal standard solution* into a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 354 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L3

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu\text{L}$

#### System suitability

**Samples:** *System suitability solution*, *Solvent blank solution*, and *Standard solution*

[NOTE—The relative retention times for anthralin, danthron, dianthrone, and *o*-nitroaniline are 1.0, 1.2, 1.7, and 2.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.3 between anthralin and danthron, *System suitability solution*

**Tailing factor:** NMT 1.5, *System suitability solution*

**Relative standard deviation:** NMT 2.0% of the ratio of the peak responses, *Standard solution*

**Interference:** No discernible signal is observed at the retention time of anthralin, *Solvent blank solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of anthralin ( $C_{14}H_{10}O_3$ ) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of anthralin to *o*-nitroaniline from the *Sample solution*

$R_S$  = peak response ratio of anthralin to *o*-nitroaniline from the *Standard solution*

$C_S$  = concentration of USP Anthralin RS in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of anthralin in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–115.0% for Ointment labeled to contain more than 0.1% of anthralin;  
90.0%–130.0% for Ointment labeled to contain 0.1% or less of anthralin

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, in a cool place. Protect from light.
- **USP REFERENCE STANDARDS** <11>  
USP Anthralin RS

### Anthrax Vaccine Adsorbed

#### DEFINITION

Anthrax Vaccine Adsorbed is a sterile, milky-white suspension made from cell-free filtrates of microaerophilic cultures of an avirulent, nonencapsulated strain of *Bacillus anthracis*. The final product contains no dead or live bacteria. The production cultures are grown in a chemically defined protein-free medium containing amino acids, vitamins, inorganic salts, and sugars. The sterile filtrate is ad-



sorbed on sterile aluminum hydroxide, concentrated 10-fold, and resuspended in sterile physiological saline containing formaldehyde with benzethonium chloride as a preservative. Sublots may be combined to produce final lots. The product meets potency requirements when tested against the U.S. Reference Standard Anthrax Vaccine, in accordance with approved procedures (guinea pig intracutaneous challenge models).

## IDENTIFICATION

### • A.

[NOTE—Perform analysis on the filtrate.]

**Trichloroacetic acid solution:** Prepare a solution of trichloroacetic acid (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water containing 100 g of trichloroacetic acid per 100 mL of the solution.

**Sample buffer:** Prepare a solution containing 141 mM tris(hydroxymethyl)aminomethane, 106 mM tris(hydroxymethyl)aminomethane hydrochloride, 0.51 mM edetate disodium, 2% (w/v) dodecyl lithium sulfate, 10% (v/v) glycerol, 0.22 mM Coomassie blue G-250, and 0.175 mM phenolsulfonphthalein. If necessary, adjust with hydrochloric acid or sodium hydroxide to a pH of 8.5.

**Running buffer:** Prepare a solution containing 25 mM tris(hydroxymethyl)aminomethane, 192 mM glycine, and 0.1% (w/v) dodecyl sodium sulfate (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water. If necessary, adjust with hydrochloric acid or sodium hydroxide to a pH of 8.5.

**Transblotting buffer:** Prepare a solution containing 12.5 mM tris(hydroxymethyl)aminomethane, 96 mM glycine, and 10% (v/v) methanol. If necessary, adjust with hydrochloric acid or sodium hydroxide to a pH of 8.0.

**Blocking buffer:** Prepare a solution containing 10 mM monobasic sodium phosphate, 150 mM sodium chloride, 5% (w/v) nonfat dry milk, and 0.05% (w/v) polysorbate 20. Adjust with sodium hydroxide to a pH of 7.4.

**Primary antibody solutions:** Prepare suitable monoclonal antibodies raised against the protective antigen (PA), the lethal factor (LF), and the edema factor (EF), respectively, of *Bacillus anthracis* in murine ascites cells, harvested, and used without further purification. Immediately before use, dilute each of the murine ascites fluids containing the monoclonal antibodies (1:1000) with the *Blocking buffer*.

**Secondary antibody solution:** Immediately before use, dissolve according to the manufacturer's instructions, if necessary, and dilute the stock horseradish peroxidase conjugated to goat anti-mouse IgG solution (1:1000) with *Blocking buffer*.

**Chromogenic visualization solution:** 150 mg/mL of 4-chloro-1-naphthol in water

**Sample solution:** Use anthrax vaccine filtrate as is.

**Analysis:** In a suitable centrifuge tube transfer (30/c) mL of the *Sample solution*, where *c* is the total protein concentration, in  $\mu\text{g/mL}$ , of the solution as determined in the test for *Total Protein*. Add  $16.5/c$  mL of *Trichloroacetic acid solution*, and incubate for at least 10 min. Centrifuge at  $9000 \times g$  for about 10 min, decant off the supernatant, and hold the tube inverted to drain on a filter paper. Dissolve the pellet in 60  $\mu\text{L}$  of *Sample buffer*, and transfer the solution to a polypropylene microfuge tube that has a lid. Close the lid tightly, secure with a lid-lock, and heat at  $100^\circ$  for 5 min. Allow the solution to cool to room temperature, and centrifuge at  $10,000 \times g$  for 15 s to collect the liquids. In a suitable device for polyacrylamide-gel electrophoresis (see *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* (1056)), add appropriate volumes of the *Running buffer* in the upper and the lower buffer chambers. Attach a 4%–20% gradient tris-glycine polyacrylamide slab gel sandwiched between two glass plates,

such that the wells for sample application are exposed to the *Running buffer* in the upper buffer chamber. Apply about 20- $\mu\text{L}$  aliquots of the treated *Sample solution* in three alternate lanes. [NOTE—Do not apply any solution in the outside lanes.] Connect the lower buffer chamber electrode to the positive terminal and the upper buffer chamber electrode to the negative terminal of a suitable power supply unit, and carry out the electrophoresis at a constant current of about 40 mA. When the dye-front is about 1 cm from the bottom of the gel (about 40 min), stop the current, and remove the gel from the gel assembly. [NOTE—Do not touch the gel with bare hands. Use gloves.]

Place three to four filter papers, cut to the size of the gel and soaked in the *Transblotting buffer*, on the anode plate of a suitable semidry electroblotter. Cut a nitrocellulose membrane to the same size as the gel plus 1–2 mm on each side, and "wet" the membrane by immersing it into the *Transblotting buffer* for about 15 s, such that there is no air bubble between the buffer and the membrane. Place the "wet" membrane immediately on the stack of filter papers, and remove all air bubbles between the membrane and filter paper by rolling a pipet, or equivalent, gently over the surface of the membrane. Place a few drops of the *Transblotting buffer* on the membrane, and then carefully place the gel on it. Gently roll a pipet, or equivalent, over the surface of the gel to ensure intimate contact between the gel and the membrane, making sure that there are no air bubbles in between. Place a filter paper cut to the size of the gel and soaked in the *Transblotting buffer*, such that there is no air bubble between the filter paper and the gel. Place two to three additional filter papers, prepared in a similar manner, on the top, and complete the transfer stack by placing the cathode plate on the top. Apply a current of about 250 mA, and continue transfer for 90 min.

Remove the membrane, and wash it quickly by immersing into water for 15 s. [NOTE—Do not touch the membrane with bare hands. Use gloves.] Cut the membrane into three strips such that each strip contains a lane containing the *Sample solution*, and mark the strips as PA, LF, and EF at the top. Place each strip in a heat-sealable bag, add 5 mL of *Blocking buffer*, and seal the bag. Incubate for 30 min with constant agitation. Open each bag, and pour out the *Blocking buffer*. Add 9 mL of the diluted *Primary antibody solution* against PA to the bag containing the strip marked PA. Similarly, add 9 mL of the diluted *Primary antibody solution* against LF and EF to the bags containing strips labeled LF and EF, respectively. Seal the bags, and incubate under agitation for 2 h at room temperature or overnight at  $2^\circ$ – $8^\circ$ . Remove the strips from the plastic bags, and place in separate plastic boxes. Add sufficient *Blocking buffer* so that each strip is completely immersed. Agitate for at least 30 min at room temperature with two changes of *Blocking buffer*. Remove the strips, and place each strip in a new heat-sealable plastic bag. Add 9 mL of the *Secondary antibody solution* to each plastic bag. Seal the bags, and incubate for 1 h at room temperature under agitation. Remove the strips from the plastic bags, and place in separate plastic boxes. Add sufficient *Blocking buffer* so that each strip is completely immersed. Agitate for at least 30 min at room temperature with two changes of the *Blocking buffer*. Transfer each strip into a new heat-sealable plastic bag, add 9 mL of *Chromogenic visualization solution* and 10  $\mu\text{L}$  of 30% (v/v) hydrogen peroxide, and seal the bags. Incubate for 30 min under agitation. Transfer the strips into separate plastic boxes, and remove the excess 4-chloro-1-naphthol by incubating with water under agitation for 10 min.

**Acceptance criteria:** Visual observation indicates a strong positive band on the strip labeled PA, a faintly



detectable band on the strip labeled LF, and no detectable band on the strip labeled EF.

## ASSAY

### • RELATIVE POTENCY

[NOTE—Perform analysis on the final product.]

**Standard solutions:** Dilute approved U.S. Reference Standard Anthrax Vaccine 1:1.6, 1:4, 1:10, and 1:25 aseptically with a sterile 0.9% sodium chloride solution.

**Sample solutions:** Dilute Anthrax Vaccine Adsorbed, final product 1:1.6, 1:4, 1:10, and 1:25 aseptically with a sterile 0.9% sodium chloride solution.

#### Analysis

**Samples:** *Standard solutions* and *Sample solutions*

Assign each dilution to a set of 12 randomly selected guinea pigs, strain Mdh:S(RA), 6 males and 6 females, each weighing 315–385 g on the day of vaccination. Inject the animals subcutaneously in the ventral abdomen with 0.5 mL of the assigned dilutions. On the 14th day post-vaccination, challenge the animals with approximately 1000 spores of *Bacillus anthracis* strain Vollum 1B, and record the deaths daily for a 10-day observation period. Record the numbers of surviving animals for each of the *Standard solutions* and the *Sample solutions* at the end of the test. Perform calculations by estimating best-fit lines for the *Standard solutions* and the *Sample solutions* using a logistic regression model that utilizes the number of animals that survived at the end of the test and the time to death for the animals that died. Evaluate statistically the lines corresponding to the *Standard solutions* and the *Sample solutions* for parallelism. Determine the common slope, and draw the parallel lines using the common slope. The relative potency of Anthrax Vaccine Adsorbed with respect to the corresponding U.S. Reference Standard Anthrax Vaccine is the antilog of the horizontal distance between the two parallel lines.

**Acceptance criteria:** The relative potency of Anthrax Vaccine Adsorbed is acceptable if it is between 0.53 and 1.79, both values inclusive.

## OTHER COMPONENTS

### • ALUMINUM (206)

[NOTE—Perform analysis on the final product.]

**Standard solutions:** Prepare as directed for *Standard Preparations* in the chapter, except to prepare solutions containing 10, 20, 30, 40, and 50 µg/mL of aluminum.

**Sample solution:** Mix Anthrax Vaccine Adsorbed, final product well, and transfer 0.2 mL to a 10-mL volumetric flask. Add 0.5 mL of concentrated sulfuric acid and 0.5 mL of concentrated nitric acid, and mix gently. Incubate at room temperature for 30 min or until the solution becomes essentially clear. Dilute with water to volume.

**Analysis:** Proceed as directed for *Procedure* in the chapter. Plot the absorbances versus the content of aluminum, in µg/mL, for the *Standard solutions*, and draw a best-fit straight line through the points using a linear regression model. Calculate the amount of aluminum in Anthrax Vaccine Adsorbed, in mg/mL.

**Acceptance criteria:** The aluminum concentration is between 0.8 and 1.5 mg/mL.

### • FORMALDEHYDE

[NOTE—Perform analysis on the final product.]

**Potassium ferricyanide solution:** Dissolve 2.5 g of potassium ferricyanide in about 100 mL of water, and mix.

**Phenylhydrazine hydrochloride solution:** Dissolve 4 g of phenylhydrazine hydrochloride in 100 mL of absolute alcohol, add 2 mL of water and mix.

**Standard stock solution:** Into a 100-mL volumetric flask containing 2.5 mL of water and 1 mL of sodium hydroxide TS 2, add 1.0 g of the formaldehyde solution to be examined, shake, and dilute with water to 100.0 mL. Determine the concentration of formaldehyde in percent (w/v) as follows. To 10.0 mL of the

solution add 30.0 mL of 0.1 N iodine VS. Mix, and add 10 mL of sodium hydroxide TS 2. After 15 min, add 25 mL of diluted sulfuric acid and 4 mL of starch TS. Titrate with 0.1 N sodium thiosulphate VS. Each 1 mL of 0.05 M iodine is equivalent to 1.501 mg of formaldehyde (CH<sub>2</sub>O).

**Standard solutions:** Dilute the *Standard stock solution* in water to obtain solutions having concentrations of 0.005%, 0.01%, and 0.02% (w/v).

**Sample solution:** Use Anthrax Vaccine Adsorbed, final product as is.

**Analysis:** To suitable glass centrifuge tubes transfer 1.0 mL each of water, the *Standard solutions*, and the *Sample solution*. To each tube, add 1.0 mL of *Potassium ferricyanide solution*, 4.0 mL of 18% (w/v) hydrochloric acid and 2.0 mL of *Phenylhydrazine hydrochloride solution*. Mix after each addition. Incubate for 50–60 min at room temperature. Centrifuge the solutions at 10,000 × g for at least 10 min, and measure absorbances of the supernatants at 540 nm using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* (857)). Plot the absorbances versus concentrations of formaldehyde, in mg/mL, in the *Standard solutions*, and draw the best-fit straight line through the points.

Calculate the percentage (w/v) of formaldehyde in the sample.

**Acceptance criteria:** The concentration of formaldehyde in Anthrax Vaccine Adsorbed is less than 0.02% (w/v).

### • BENZETHONIUM CHLORIDE

[NOTE—Perform analysis on the final product.]

**Citrate buffer:** 25 g of citric acid monohydrate in about 60 mL of water, and adjust with a solution of sodium hydroxide to a pH of 4.5. Transfer the solution to a 100-mL volumetric flask. Dilute with water to volume and mix.

**Dye solution:** 50 mg of 2',4',5',7'-tetrabromofluorescein in about 100 mL of water, and mix. Dilute 1 mL of this solution with water to 100 mL.

**Docusate sodium solution:** 50 µg/mL of docusate sodium

**Standard solution A:** 0.5 g of benzethonium chloride in a 100-mL volumetric flask, dissolve in 60 mL of water, dilute with water to volume, and mix.

**Standard solutions B, C, D, and E:** Dilute *Standard solution A* with water to obtain solutions having concentrations of 0.001%, 0.002%, 0.003%, and 0.004% (w/v), respectively.

**Sample solution:** Use Anthrax Vaccine Adsorbed, final product as is.

#### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** *Docusate sodium solution*

**Endpoint detection:** Visual

**Analysis:** Transfer 4.0 mL each of *Standard solutions B, C, D, and E* and the *Sample solution* to suitable glass centrifuge tubes. Add 1.0 mL of *Citrate buffer* and 0.4 mL of the *Dye solution* to each tube. Add 4.0 mL of 1,1,2,2-tetrachloroethane to each tube, and vigorously mix on a vortex mixer for 1 min. Centrifuge at about 1000 × g for at least 15 min to separate the organic layer from the aqueous layer. Transfer 2.0 mL of the organic layer from the tubes to another set of glass tubes. Add 4.0 mL of water and 0.5 mL of *Citrate buffer* to each tube, and mix on a vortex mixer for about 1 min. Titrate the benzethonium chloride-dye complex in each tube with the *Titrant* to the colorimetric endpoint indicated by the disappearance of the pink color of the organic layer. [NOTE—Vigorously mix the solution on a vortex mixer after each addition of the *Docusate sodium solution*.] Plot the volumes of *Docusate sodium solution* required versus the concentrations of benzethonium chloride in *Standard solutions B, C, D, and E*, and draw a best-fit straight line through the points. Determine the



concentration of benzethonium chloride in the *Sample solution* from the volume of *Titrant* required to titrate the *Sample solution*.

**Acceptance criteria:** The concentration of benzethonium chloride in Anthrax Vaccine Adsorbed is between 0.0015% and 0.0030% (w/v).

## SPECIFIC TESTS

### • 83 kDa PROTEIN

[NOTE—Perform tests on the filtrate.]

**Trichloroacetic acid solution, Sample buffer, Running buffer, and Sample solution:** Prepare as directed in Identification test A.

**Staining solution:** Prepare a solution of Coomassie blue G-250 having a concentration of 1.25 g/L in a mixture of methanol, acetic acid, and water (4:1:5, v/v).

**Protein molecular weight standard solution:** Reconstitute a vial of protein molecular weight standard mixture containing proteins of molecular weights at least in the range of 14–200 kDa, according to manufacturer's instruction. Dilute the solution with *Sample buffer* such that the concentration of each protein in the solution is about 0.5 µg/µL.

**Analysis:** In a suitable centrifuge tube transfer (10/c) mL of the *Sample solution*, where *c* is the total protein concentration, in µg/mL, of the solution as determined by the test for *Total Protein*. Add 5.5/c mL of *Trichloroacetic acid solution*, and incubate for at least 10 min. Centrifuge at 9000 × *g* for about 10 min, decant off the supernatant, and hold the tube inverted to drain on a filter paper. Dissolve the pellet in 20 µL of *Sample buffer*, and transfer the solution to a polypropylene microfuge tube with a lid. Transfer 20 µL of *Protein molecular weight standard solution* to another polypropylene microfuge tube with a lid. Close the lids tightly, secure with lid-locks, and heat both solutions at 100° for 5 min. Allow the solutions to cool to room temperature, and centrifuge at 10,000 × *g* for 15 s to collect the liquids. Apply the solutions to two consecutive lanes of a 4%–20% gradient tris-glycine polyacrylamide slab gel [NOTE—Do not apply any solution in the outside lanes.], and electrophorese as directed in *Identification* (see *Biotechnology-Derived Articles—Polyacrylamide Gel Electrophoresis* (1056)). When the dye-front is about 1 cm from the bottom of the gel (about 40 min), stop the current, and remove the gel from the gel assembly. Soak the gel in a suitable volume of the *Staining solution* for at least 1 h, such that the gel is completely immersed in the *Staining solution* during staining. [NOTE—Do not touch the gel with bare hands. Use disposable gloves.] Destain the gel with a large volume of water under constant agitation with repeated changes of water until the background of the gel is completely color free. Using the molecular weights of the proteins in *Protein molecular weight standard solution*, identify the band corresponding to the PA (MW about 83 kDa) in the *Sample solution* lane. [NOTE—This band is also the predominant band in the lane of the *Sample solution*.] Scan the gel, and determine the relative amount (by peak area) of the 83 kDa band by densitometry in the lane of the *Sample solution*.

**Acceptance criteria:** The content of 83 kDa band is NLT 35% of the total peak area.

### • TOTAL PROTEIN

[NOTE—Perform tests on the filtrate.]

**Standard solution A:** Prepare a solution of albumin bovine serum (see *Reagents, Indicators, and Solutions—Reagent Specifications*) in water to obtain a known concentration of 2.0 mg/mL.

**Standard solutions B, C, D, and E:** Dilute *Standard solution A* with water to obtain solutions having protein concentrations of 4, 8, 16, and 24 µg/mL, respectively.

**Sample solution:** Use anthrax vaccine filtrate as is.

### Analysis

(See *Biotechnology-Derived Articles—Total Protein Assay* (1057), Method 3.)

To a series of test tubes transfer 800 µL each of *Standard solutions B, C, D, and E* and the *Sample solution*. Also transfer 800 µL of water to be used as the blank. Add 200 µL of Coomassie blue G-250 dye solution (see *Reagents, Indicators, and Solutions—Reagent Specifications*) to each tube, and mix without foaming. Determine absorbances of the solutions at 595 nm using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* (857)), using the blank to set the instrument to zero.

[NOTE—Do not use quartz (silica) spectrophotometer cells; the dye binds to silica.]

Construct a standard curve by plotting the absorbances versus protein concentrations, in µg/mL, of *Standard solutions B, C, D, and E* and by drawing a best-fit straight line using the linear regression method. From the standard curve, determine the total protein concentration of the *Sample solution* using the absorbance value.

**Acceptance criteria:** The protein concentration is between 5 and 20 µg/mL.

- **SAFETY:** It meets the requirements when tested as directed in *Biological Reactivity Tests, In Vivo* (88), *Safety Tests—Biologicals*.

[NOTE—Perform tests on final product.]

- **STERILITY TESTS (71):** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*.

[NOTE—Perform tests on final product.]

- **pH (791):** 7.5–8.5

### • SODIUM CHLORIDE

[NOTE—Perform tests on the final product.]

**Standard solutions A and B:** Prepare two solutions of sodium chloride in water having concentrations of 0.2 mM and 2.0 mM, respectively.

**Sample solution:** Transfer 0.5 mL of Anthrax Vaccine Adsorbed, final product to a 50-mL volumetric flask. Dilute with water to volume.

**Analysis:** Determine the voltage readings of *Standard solutions A and B* and the *Sample solution* using an ion-specific electrode specific for the chloride ion electrically coupled with a standard silver-silver chloride reference electrode. Plot the voltage readings versus concentration of chloride, in mg/mL, for *Standard solutions A and B*, and draw a straight line joining the points.

Calculate the concentration of chloride ion in the *Sample solution* from the voltage reading. Assuming that the chloride ion comes entirely from sodium chloride, calculate the concentrations of sodium chloride in the *Sample solution*.

**Acceptance criteria:** The concentration of sodium chloride in Anthrax Vaccine Adsorbed is between 0.75% and 0.95% (w/v).

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in multiple-dose tight Type I glass containers. Store at a temperature between 2° and 8°. Do not freeze.
- **LABELING:** Label it to state that it is to be well shaken before use and that it is not to be frozen.
- **EXPIRATION DATE:** The expiration date is 18 months from the date of manufacture.



## Anticoagulant Citrate Dextrose Solution

### DEFINITION

Anticoagulant Citrate Dextrose Solution is a sterile solution of Citric Acid, Sodium Citrate, and Dextrose in Water for Injection. It contains no antimicrobial agents, and in each 1000 mL it contains:

	Solution A	Solution B
Total Citrate, expressed as anhydrous citric acid ( $C_6H_8O_7$ )	NLT 20.59 g NMT 22.75 g	NLT 12.37 g NMT 13.67 g
Dextrose ( $C_6H_{12}O_6 \cdot H_2O$ )	NLT 23.28 g NMT 25.73 g	NLT 13.96 g NMT 15.44 g
Sodium (Na)	NLT 4.90 g NMT 5.42 g	NLT 2.94 g NMT 3.25 g

Prepare Anticoagulant Citrate Dextrose Solution as follows.

	Solution A	Solution B
Citric Acid (anhydrous)	7.3 g	4.4 g
Sodium Citrate (dihydrate)	22.0 g	13.2 g
Dextrose (monohydrate)	24.5 g	14.7 g
Water for Injection, a sufficient quantity to make	1000 mL	1000 mL

Dissolve the ingredients, and mix. Filter the solution until clear, place immediately in suitable containers, and sterilize.

If desired, 8 g and 4.8 g of monohydrated citric acid may be used instead of the indicated, respective amounts of anhydrous citric acid; 19.3 g and 11.6 g of anhydrous sodium citrate may be used instead of the indicated, respective amounts of dihydrated sodium citrate; and 22.3 g and 13.4 g of anhydrous dextrose may be used instead of the indicated, respective amounts of monohydrated dextrose.

### IDENTIFICATION

#### A. DEXTROSE

**Analysis:** Add a few drops of solution (1 in 20) to 5 mL of hot alkaline cupric tartrate TS.

**Acceptance criteria:** A copious red precipitate of cuprous oxide is formed.

#### B. IDENTIFICATION TESTS—GENERAL, Citrate (191):

Meets the requirements when concentrated to one-half its volume

#### C. IDENTIFICATION TESTS—GENERAL, Sodium (191):

Meets the requirements when concentrated to one-half its volume

### ASSAY

#### TOTAL CITRATE

Mobile phase, Standard preparation 1, and Chromatographic system: Proceed as directed in Assay for Citric Acid/Citrate and Phosphate (345).

**Sample solution:** Pipet 5 mL of Solution into a suitable volumetric flask, and proceed as directed in Assay for Citric Acid/Citrate and Phosphate (345), Assay Preparation for Citric Acid/Citrate Assay.

#### Analysis

**Samples:** Standard preparation 1 and Sample solution Proceed as directed in Assay for Citric Acid/Citrate and Phosphate (345), Procedure.

Calculate the quantity, in g, of anhydrous citric acid ( $C_6H_8O_7$ ) in the volume of Solution taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times F \times D$$

$r_U$  = peak area of citrate from the Sample solution  
 $r_S$  = peak area of citrate from Standard preparation 1

$C_S$  = concentration of citrate in Standard preparation 1 ( $\mu\text{g/mL}$ )

$M_{r1}$  = molecular weight of anhydrous citric acid, 192.12

$M_{r2}$  = molecular weight of citrate ( $C_6H_5O_7$ ), 189.10

$F$  = conversion factor, 0.000001 g/ $\mu\text{g}$

$D$  = dilution factor

**Acceptance criteria:** Each 1000 mL should contain 20.59–22.75 g in Solution A and 12.37–13.67 g in Solution B of total citrate expressed as anhydrous citric acid.

#### SODIUM

**Solution A:** Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, add water to volume, and mix. This solution contains 15 mEq/1000 mL of lithium.

**Standard solution:** Transfer 8.18 g of sodium chloride, previously dried at 105° for 2 h to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 140 mEq/1000 mL of sodium. Transfer 50  $\mu\text{L}$  of this solution to a 10-mL volumetric flask, dilute with Solution A to volume, and mix.

**Sample solution:** Transfer 25 mL of Solution to a 50-mL volumetric flask, and dilute with water to volume.

Transfer 50  $\mu\text{L}$  of this solution to a 10-mL volumetric flask, dilute with Solution A to volume, and mix.

#### Analysis

**Samples:** Standard solution and Sample solution

Using a suitable flame photometer, adjusted to read zero with Solution A, concomitantly determine the sodium flame emission readings for the Standard solution and the Sample solution at the wavelength of maximum emission at 589 nm.

Calculate the quantity, in g, of sodium (Na) in 1000 mL of Solution taken:

$$\text{Result} = (r_U/r_S) \times (A_r/M_r) \times W \times F$$

$r_U$  = sodium emission readings from the Sample solution

$r_S$  = sodium emission readings from the Standard solution

$A_r$  = atomic weight of sodium, 22.99

$M_r$  = molecular weight of sodium chloride, 58.44

$W$  = weight of sodium chloride taken to make the Standard solution, 8.18 g

$F$  = conversion factor, 2

**Acceptance criteria:** Each 1000 mL should contain 4.90–5.42 g in Solution A and 2.94–3.25 g in Solution B of sodium.

#### DEXTROSE

**Analysis:** Determine the angular rotation of Solution in a suitable polarimeter tube (see Optical Rotation (781)). Where Solution is labeled to contain anhydrous dextrose, calculate the percentage, in g/100 mL, of anhydrous dextrose ( $C_6H_{12}O_6$ ) in the portion of Solution taken:

$$\text{Result} = (100/F) \times A \times R$$

$F$  = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

$A$  = 100 mm divided by the length of the polarimeter tube (mm)

$R$  = observed rotation (°)

Where Solution is labeled to contain dextrose monohydrate, calculate the percentage, in g/100 mL, of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) in the portion of Solution taken:

$$\text{Result} = (100/F) \times (M_{r1}/M_{r2}) \times A \times R$$

$F$  = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

$M_{r1}$  = molecular weight for dextrose monohydrate, 198.17



- $M_{r2}$  = molecular weight for anhydrous dextrose, 180.16  
 $A$  = 100 mm divided by the length of the polarimeter tube (mm)  
 $R$  = observed rotation (°)  
**Acceptance criteria:** Each 1000 mL should contain 23.28–25.73 g in Solution A and 13.96–15.44 g in Solution B of dextrose monohydrate.

**IMPURITIES**

- **CHLORIDE AND SULFATE, Chloride (221):** A 10-mL portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.0035%).

**SPECIFIC TESTS**

- **PH (791):** 4.5–5.5
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products (1)*
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 5.56 USP Endotoxin Units/mL.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose containers of colorless, transparent Type I or Type II glass or of a suitable plastic material (see *Transfusion and Infusion Assemblies and Similar Medical Devices (161)*).
- **LABELING:** Label to indicate the number of mL of Solution required per 100 mL of whole blood or the number of mL of Solution required per volume of whole blood to be collected.
- **USP REFERENCE STANDARDS (11)**  
 USP Citric Acid RS  
 USP Endotoxin RS

## Anticoagulant Citrate Phosphate Dextrose Solution

**DEFINITION**

Anticoagulant Citrate Phosphate Dextrose Solution is a sterile solution of Citric Acid, Sodium Citrate, Monobasic Sodium Phosphate, and Dextrose in Water for Injection. It contains, in each 1000 mL, NLT 2.11 g and NMT 2.33 g of monobasic sodium phosphate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ); NLT 24.22 g and NMT 26.78 g of dextrose ( $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$ ); NLT 19.16 g and NMT 21.18 g of total citrate, expressed as anhydrous citric acid ( $\text{C}_6\text{H}_8\text{O}_7$ ); and NLT 6.21 g and NMT 6.86 g of Sodium (Na). It contains no antimicrobial agents.

Prepare Anticoagulant Citrate Phosphate Dextrose Solution as follows.

Citric Acid (anhydrous)	2.99 g
Sodium Citrate (dihydrate)	26.3 g
Monobasic Sodium Phosphate (monohydrate; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ )	2.22 g
Dextrose ( $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{H}_2\text{O}$ )	25.5 g
Water for Injection, a sufficient quantity to make	1000 mL

Dissolve the ingredients, and mix. Filter the solution until clear, place immediately in suitable containers, and sterilize.

If desired, 3.27 g of monohydrated citric acid may be used instead of the indicated amount of anhydrous citric acid; 23.06 g of anhydrous sodium citrate may be used instead of the indicated amount of dihydrated sodium citrate; 1.93 g of anhydrous monobasic sodium phosphate may be used instead of the indicated amount of monohydrated monobasic sodium phosphate; and 23.2 g of anhydrous dextrose may be used instead of the indicated amount of monohydrated dextrose.

**IDENTIFICATION**

- **A. DEXTROSE**  
**Analysis:** Add a few drops of solution (1 in 20) to 5 mL of hot alkaline cupric tartrate TS.  
**Acceptance criteria:** A copious red precipitate of cuprous oxide is formed.
- **B. IDENTIFICATION TESTS—GENERAL, Phosphate (191):** Meets the requirements
- **C. IDENTIFICATION TESTS—GENERAL, Citrate (191):** Meets the requirements when concentrated to one-half its volume
- **D. IDENTIFICATION TESTS—GENERAL, Sodium (191):** Meets the requirements when concentrated to one-half its volume

**ASSAY****• TOTAL CITRATE AND TOTAL PHOSPHATE**

**Mobile phase, Standard preparation 2, and Chromatographic system:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate (345)*.

**Sample solution for total citrate:** Pipet 10 mL of Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate (345)*, *Assay Preparation for Citric Acid/Citrate Assay*.

**Sample solution for total phosphate:** Pipet 5 mL of Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate (345)*, *Assay Preparation for Phosphate Assay*.

**Analysis:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate (345)*, *Procedure*.

Calculate the quantity, in g, of anhydrous citric acid ( $\text{C}_6\text{H}_8\text{O}_7$ ) in the volume of Solution taken:

$$\text{Result} = (r_u/r_s) \times C_s \times (M_{r1}/M_{r2}) \times F \times D$$

$r_u$  = peak area of citrate from the *Sample solution for total citrate*

$r_s$  = peak area of citrate from *Standard preparation 2*

$C_s$  = concentration of citrate in *Standard preparation 2* ( $\mu\text{g/mL}$ )

$M_{r1}$  = molecular weight of anhydrous citric acid ( $\text{C}_6\text{H}_8\text{O}_7$ ), 192.12

$M_{r2}$  = molecular weight of citrate ( $\text{C}_6\text{H}_5\text{O}_7$ ), 189.10

$F$  = conversion factor, 0.000001 g/ $\mu\text{g}$

$D$  = dilution factor

**Acceptance criteria:** Each 1000 mL of Solution should contain 19.16 g–21.18 g of total citrate expressed as anhydrous citric acid.

**Analysis:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate (345)*, *Procedure*.

Calculate the quantity of phosphate, in g, expressed as monobasic sodium phosphate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ), in the volume of Solution taken:

$$\text{Result} = (r_u/r_s) \times C_s \times (M_{r1}/M_{r2}) \times F \times D$$

$r_u$  = peak area of phosphate from the *Sample solution for total phosphate*

$r_s$  = peak area of phosphate from *Standard preparation 2*

$C_s$  = concentration of phosphate in *Standard preparation 2* ( $\mu\text{g/mL}$ )

$M_{r1}$  = molecular weight of monobasic sodium phosphate monohydrate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ), 137.99

$M_{r2}$  = molecular weight of phosphate ( $\text{PO}_4$ ), 94.97

$F$  = conversion factor, 0.000001 g/ $\mu\text{g}$

$D$  = dilution factor

**Acceptance criteria:** Each 1000 mL of Solution should contain 2.11–2.33 g of monobasic sodium phosphate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ).



**• DEXTROSE**

**Sample:** 5.0 mL of Solution

**Analysis:** Tare a clean, medium-porosity filtering crucible containing several carborundum boiling chips or glass beads. Pipet 50 mL of freshly mixed alkaline cupric tartrate TS into a 400-mL beaker. Add the boiling chips or glass beads from the tared crucible, 45 mL of water, and 5.0 mL of Solution to the beaker. Heat the beaker and contents over a burner that has been adjusted to cause boiling of the solution to start in 3.5–4 min. Boil the solution for 2 min, accurately timed, and filter immediately through the tared crucible, taking care to transfer all of the boiling chips or glass beads to the crucible. Wash the precipitate with hot water and 10 mL of alcohol. Dry the crucible and contents at 110° to constant weight. Perform a blank determination, and correct the weight of the precipitate from the sample for any precipitate obtained in the blank.

Each mg of cuprous oxide precipitate of the substance under assay is equivalent to 0.496 mg of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ).

**Acceptance criteria:** Each 1000 mL of Solution should contain 24.22–26.78 g of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ).

**• SODIUM**

**Solution A:** Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, add water to volume, and mix. This solution contains 15 mEq/1000 mL of lithium.

**Standard solution:** Transfer 8.18 g of sodium chloride, previously dried at 105° for 2 h to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 140 mEq/1000 mL of sodium. Transfer 50  $\mu$ L of this solution to a 10-mL volumetric flask, dilute with Solution A to volume, and mix.

**Sample solution:** Transfer 25 mL of Solution to a 50-mL volumetric flask, and dilute with water to volume. Transfer 50  $\mu$ L of this solution to a 10-mL volumetric flask, dilute with Solution A to volume, and mix.

**Analysis**

**Samples:** Standard solution and Sample solution

Using a suitable flame photometer, adjusted to read zero with Solution A, concomitantly determine the sodium flame emission readings for the Standard solution and the Sample solution at the wavelength of maximum emission at 589 nm.

Calculate the quantity, in g, of sodium (Na) in 1000 mL of Solution taken:

$$\text{Result} = (r_u/r_s) \times (A_r/M_r) \times W \times F$$

- $r_u$  = sodium emission readings from the Sample solution  
 $r_s$  = sodium emission readings from the Standard solution  
 $A_r$  = atomic weight of sodium, 22.99  
 $M_r$  = molecular weight of sodium chloride, 58.44  
 $W$  = weight of sodium chloride taken to make the Standard solution, 8.18 g  
 $F$  = conversion factor, 2

**Acceptance criteria:** Each 1000 mL of Solution should contain 6.21–6.86 g of sodium.

**IMPURITIES**

- CHLORIDE AND SULFATE, Chloride (221):** A 10-mL portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.0035%).

**SPECIFIC TESTS**

- PH (791):** 5.0–6.0
- BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 5.56 USP Endotoxin Units/mL.
- OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in single-dose containers of colorless, transparent Type I or Type II glass, or of a suitable plastic material (see *Transfusion and Infusion Assemblies and Similar Medical Devices* (161)).
- LABELING:** Label it to indicate the number of mL of Solution required per 100 mL of whole blood or the number of mL of Solution required per volume of whole blood to be collected.
- USP REFERENCE STANDARDS (11)**
  - USP Citric Acid RS
  - USP Endotoxin RS

## Anticoagulant Citrate Phosphate Dextrose Adenine Solution

**DEFINITION**

Anticoagulant Citrate Phosphate Dextrose Adenine Solution is a sterile solution of Citric Acid, Sodium Citrate, Monobasic Sodium Phosphate, Dextrose, and Adenine in Water for Injection. It contains, in each 1000 mL, NLT 2.11 g and NMT 2.33 g of monobasic sodium phosphate ( $NaH_2PO_4 \cdot H_2O$ ); NLT 30.30 g and NMT 33.50 g of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ); NLT 19.16 g and NMT 21.18 g of total citrate, expressed as citric acid, anhydrous ( $C_6H_8O_7$ ); NLT 6.21 g and NMT 6.86 g of sodium (Na); and NLT 0.247 g and NMT 0.303 g of adenine ( $C_5H_5N_5$ ). It contains no antimicrobial agents.

Prepare Anticoagulant Citrate Phosphate Dextrose Adenine Solution as follows.

Citric Acid (anhydrous)	2.99 g
Sodium Citrate (dihydrate)	26.3 g
Monobasic Sodium Phosphate (monohydrate; $NaH_2PO_4 \cdot H_2O$ )	2.22 g
Dextrose (monohydrate)	31.9 g
Adenine ( $C_5H_5N_5$ )	0.275 g
Water for Injection, a sufficient quantity to make	1000 mL

Dissolve the ingredients, and mix. Filter the solution until clear, place immediately in suitable containers, and sterilize.

If desired, 3.27 g of monohydrated citric acid may be used instead of the indicated amount of anhydrous citric acid; 23.06 g of anhydrous sodium citrate may be used instead of the indicated amount of dihydrated sodium citrate; 1.93 g of anhydrous monobasic sodium phosphate may be used instead of the indicated amount of monohydrated monobasic sodium phosphate; and 29.0 g of anhydrous dextrose may be used instead of the indicated amount of monohydrated dextrose.

**ASSAY****• TOTAL CITRATE AND TOTAL PHOSPHATE**

**Mobile phase, Standard preparation 2, and Chromatographic system:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345).

**Sample solution for total citrate:** Pipet 10 mL of Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345), *Assay Preparation for Citric Acid/Citrate Assay*.

**Sample solution for total phosphate:** Pipet 5 mL of Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345), *Assay Preparation for Phosphate Assay*.

**Analysis**

**Samples:** Standard preparation 2 and Sample solution for total citrate

Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345), *Procedure*.



Calculate the quantity, in g, of anhydrous citric acid ( $C_6H_8O_7$ ) in the volume of Solution taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times F \times D$$

- $r_U$  = peak area of citrate from the *Sample solution* for total citrate  
 $r_S$  = peak area of citrate from *Standard preparation 2*  
 $C_S$  = concentration of citrate in *Standard preparation 2* ( $\mu\text{g/mL}$ )  
 $M_{r1}$  = molecular weight of anhydrous citric acid, 192.12  
 $M_{r2}$  = molecular weight of citrate ( $C_6H_5O_7$ ), 189.10  
 $F$  = conversion factor, 0.000001 g/ $\mu\text{g}$   
 $D$  = dilution factor

**Acceptance criteria:** Each 1000 mL of Solution should contain NLT 19.16 g and NMT 21.18 g of total citrate expressed as anhydrous citric acid ( $C_6H_8O_7$ ).

#### Analysis

**Samples:** *Standard preparation 2* and *Sample solution* for total phosphate

Calculate the quantity of phosphate, in g, expressed as monobasic sodium phosphate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ), in the volume of Solution taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times F \times D$$

- $r_U$  = peak area of phosphate from the *Sample solution* for total phosphate  
 $r_S$  = peak area of phosphate from *Standard preparation 2*  
 $C_S$  = concentration of phosphate in *Standard preparation 2* ( $\mu\text{g/mL}$ )  
 $M_{r1}$  = molecular weight of monobasic sodium phosphate monohydrate, 137.99  
 $M_{r2}$  = molecular weight of phosphate ( $\text{PO}_4$ ), 94.97  
 $F$  = conversion factor, 0.000001 g/ $\mu\text{g}$   
 $D$  = dilution factor

**Acceptance criteria:** Each 1000 mL of Solution should contain 2.11–2.33 g of monobasic sodium phosphate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ).

#### • SODIUM

**Solution A:** Transfer 1.04 g of lithium nitrate to a 1000-mL volumetric flask, add a suitable nonionic surfactant, add water to volume, and mix. This solution contains 15 mEq/1000 mL of lithium.

**Standard solution:** Transfer 8.18 g of sodium chloride, previously dried at  $105^\circ$  for 2 h to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 140 mEq/1000 mL of sodium. Transfer 50  $\mu\text{L}$  of this solution to a 10-mL volumetric flask, dilute with *Solution A* to volume, and mix.

**Sample solution:** Transfer 25 mL of Solution to a 50-mL volumetric flask, dilute with water to volume, and mix. Transfer 50  $\mu\text{L}$  of this solution to a 10-mL volumetric flask, dilute with *Solution A* to volume, and mix.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Using a suitable flame photometer, adjusted to read zero with *Solution A*, concomitantly determine the sodium flame emission readings for the *Standard solution* and the *Sample solution* at the wavelength of maximum emission at 589 nm.

Calculate the quantity, in g, of sodium (Na) in 1000 mL of Solution taken:

$$\text{Result} = (r_U/r_S) \times (A_r/M_r) \times W \times F$$

- $r_U$  = sodium emission readings from the *Sample solution*  
 $r_S$  = sodium emission readings from the *Standard solution*  
 $A_r$  = atomic weight of sodium, 22.99

- $M_r$  = molecular weight of sodium chloride, 58.44  
 $W$  = weight of sodium chloride taken to make the *Standard solution*, 8.18 g  
 $F$  = conversion factor, 2

**Acceptance criteria:** Each 1000 mL of Solution should contain 6.21 g–6.86 g of sodium.

#### • DEXTROSE

**Sample:** 5 mL of Solution

**Analysis:** Tare a clean, medium-porosity filtering crucible containing several carborundum boiling chips or glass beads. Pipet 50 mL of freshly mixed alkaline cupric tartrate TS into a 400-mL beaker. Add the boiling chips or glass beads from the tared crucible, 45 mL of water, and 5.0 mL of Solution to the beaker. Heat the beaker and contents over a burner that has been adjusted to cause boiling of the solution to start in 3.5–4 min. Boil the solution for 2 min, accurately timed, and filter immediately through the tared crucible, taking care to transfer all of the boiling chips or glass beads to the crucible. Wash the precipitate with hot water and 10 mL of alcohol. Dry the crucible and contents at  $110^\circ$  to constant weight. Perform a blank determination, and make any necessary correction.

Each mg of cuprous oxide precipitate obtained is equivalent to 0.496 mg of dextrose ( $C_6H_{12}O_6 \cdot \text{H}_2\text{O}$ ).

**Acceptance criteria:** Each 1000 mL of Solution should contain 30.30–33.50 g of dextrose ( $C_6H_{12}O_6 \cdot \text{H}_2\text{O}$ ).

#### • ADENINE

**Mobile phase:** Dissolve 3.45 g of ammonium dihydrogen phosphate in 950 mL of water, add 10 mL of glacial acetic acid, dilute with water to 1000 mL, and mix. Pass the solution through a membrane filter with a 1- $\mu\text{m}$  or finer pore size, and degas.

**System suitability solution:** 0.275 mg/mL of each USP Adenine RS and purine in dilute hydrochloric acid (1 in 120)

**Standard solutions:** Place quantities of USP Adenine RS in dilute hydrochloric acid (1 in 120) in three separate volumetric flasks, and dilute with the dilute hydrochloric acid solution to volume to obtain *Standard solutions* having known concentrations of 0.25, 0.275, and 0.30 mg of adenine per mL, respectively. Protect from light.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm  $\times$  30-cm stainless steel; packing L9

**Flow rate:** 2.0 mL/min

**Injection volume:** 20  $\mu\text{L}$

#### System suitability

**Sample:** *System suitability solution* (NLT four injections)

#### Suitability requirements

**Resolution:** NLT 3.0 between adenine and purine

**Relative standard deviation:** NMT 2.5% for adenine peak and NMT 2.0% for the retention time of adenine peak

#### Analysis

**Samples:** *Standard solutions* and *Solution*  
 Plot the responses against the concentrations, in mg, of USP Adenine RS per mL of the *Standard solutions*. Calculate the quantity, in mg, of adenine ( $C_5H_5N_5$ ) in each mL of the Solution taken as the value read directly from the Standard curve corresponding to the response obtained from the portion of the Solution chromatographed.

**Acceptance criteria:** Each 1000 mL of Solution should contain 0.247–0.303 g of adenine ( $C_5H_5N_5$ ).

#### IMPURITIES

- **CHLORIDE AND SULFATE, Chloride (221):** A 10-mL portion shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.0035%).



**SPECIFIC TESTS**

- **PH (791):** 5.0–6.0
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 5.56 USP Endotoxin Units/mL.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, of colorless, transparent, Type I or Type II glass, or of a suitable plastic material (see *Transfusion and Infusion Assemblies and Similar Medical Devices* (161)).
- **LABELING:** Label to indicate the number of mL of solution required per 100 mL of whole blood or the number of mL of solution required per volume of whole blood to be collected.
- **USP REFERENCE STANDARDS (11)**  
USP Adenine RS  
USP Citric Acid RS  
USP Endotoxin RS

**Anticoagulant Sodium Citrate Solution****DEFINITION**

Anticoagulant Sodium Citrate Solution is a sterile solution of Sodium Citrate in Water for Injection. It contains, in each 100 mL, NLT 3.80 g and NMT 4.20 g of sodium citrate dihydrate ( $C_6H_5Na_3O_7 \cdot 2H_2O$ ). It contains no antimicrobial agents.

Prepare Anticoagulant Sodium Citrate Solution as follows.

Sodium Citrate (dihydrate)	40 g
Water for Injection, a sufficient quantity to make	1000 mL

[NOTE—Anhydrous sodium citrate (35.1 g) may be used instead of the dihydrate.]

Dissolve the *Sodium Citrate* in sufficient *Water for Injection* to make 1000 mL, and filter until clear. Place the solution in suitable containers, and sterilize.

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Citrate (191):** Meets the requirements when evaporated to a concentration of 1 in 20
- **B. IDENTIFICATION TESTS—GENERAL, Sodium (191):** Meets the requirements when evaporated to a concentration of 1 in 20

**ASSAY****PROCEDURE**

**Mobile phase, Standard preparation 1, and Chromatographic system:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345).

**Sample solution:** Pipet 10 mL of Solution into a suitable volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345), *Assay Preparation for Citric Acid/Citrate Assay*.

**Analysis**

**Samples:** *Standard preparation 1* and *Sample solution*  
Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345), *Procedure*.

Calculate the quantity, in g, of sodium citrate dihydrate ( $C_6H_5Na_3O_7 \cdot 2H_2O$ ) in the volume of Solution taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times D \times F$$

- $r_U$  = peak area of citrate from the *Sample solution*  
 $r_S$  = peak area of citrate from *Standard preparation 1*  
 $C_S$  = concentration of citrate in *Standard preparation 1* ( $\mu\text{g/mL}$ )

$M_{r1}$  = molecular weight of sodium citrate dihydrate, 294.10

$M_{r2}$  = molecular weight of citrate ( $C_6H_5O_7$ ), 189.10

$D$  = dilution factor

$F$  = conversion factor, 0.000001 g/ $\mu\text{g}$

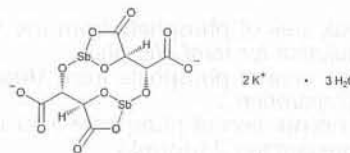
**Acceptance criteria:** In each 100 mL, 3.80–4.20 g of sodium citrate dihydrate ( $C_6H_5Na_3O_7 \cdot 2H_2O$ )

**SPECIFIC TESTS**

- **PH (791):** 6.4–7.5
- **BACTERIAL ENDOTOXINS TEST (85):** Contains NMT 5.56 USP Endotoxin Units/mL
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I or Type II glass.
- **USP REFERENCE STANDARDS (11)**  
USP Citric Acid RS  
USP Endotoxin RS

**Antimony Potassium Tartrate**

$C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$  667.87

$C_8H_4K_2O_{12}Sb_2$  613.82

Antimonate(2-), bis[ $\mu$ -[2,3-dihydroxybutanedioato(4-)- $O^1, O^2: O^3, O^4$ ]-di-, dipotassium, trihydrate, stereoisomer; Dipotassium bis[ $\mu$ -L-(+)-tartrato(4-)]diantimonate(2-) trihydrate [28300-74-5].  
Anhydrous [11071-15-1].

**DEFINITION**

Antimony Potassium Tartrate contains NLT 99.0% and NMT 103.0% of antimony potassium tartrate ( $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$ ).

**IDENTIFICATION****A.**

**Sample:** An appropriate quantity

**Analysis:** Heat the *Sample* to redness.

**Acceptance criteria:** It chars, emits an odor resembling that of burning sugar, and leaves a blackened residue. This residue has an alkaline reaction, and when a small fragment of it is held in a nonluminous flame, the flame is tinted violet.

**B.**

**Sample solution:** Antimony Potassium Tartrate (1 in 20) in water acidified with hydrochloric acid

**Analysis:** Add hydrogen sulfide TS to the *Sample solution*.

**Acceptance criteria:** It yields an orange-red precipitate, which is soluble in ammonium sulfide TS and in 1 N sodium hydroxide.

- **C. IDENTIFICATION TESTS—GENERAL, Tartrate (191):** Meets the requirements

**ASSAY****PROCEDURE**

**Sample:** 500 mg

**Analysis:** Dissolve the *Sample* in 50 mL of water, add 5 g of potassium sodium tartrate, 2 g of sodium borate, and 3 mL of starch TS, and immediately titrate with 0.1 N iodine VS to the production of a persistent blue



color. Each mL of 0.1 N iodine is equivalent to 16.70 mg of antimony potassium tartrate ( $C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$ ).

Acceptance criteria: 99.0%–103.0%

### IMPURITIES

#### • ARSENIC

**Sample solution:** Dissolve 100 mg in 5 mL of hydrochloric acid. Add 10 mL of a recently prepared solution of 20 g of stannous chloride in 30 mL of hydrochloric acid.

**Blank:** Add 5 mL of hydrochloric acid to 10 mL of a recently prepared solution of 20 g of stannous chloride in 30 mL of hydrochloric acid.

**Analysis:** Transfer the *Sample solution* to a color-comparison tube, and allow to stand for 30 min.

**Acceptance criteria:** NMT 0.015%; viewed downward over a white surface, the color of the solution appears no deeper than that of the *Blank* to which has been added 15 µg of arsenic.

#### • LEAD (251): NMT 20 ppm

### SPECIFIC TESTS

#### • COMPLETENESS OF SOLUTION (641)

**Sample:** 750 mg

**Solvent:** Water

**Acceptance criteria:** Meets the requirements

#### • LOSS ON DRYING (731)

**Analysis:** Dry at 105° to constant weight.

**Acceptance criteria:** NMT 2.7%

#### • ACIDITY OR ALKALINITY

**Sample solution:** Dissolve 1.0 g in 50 mL of carbon dioxide-free water.

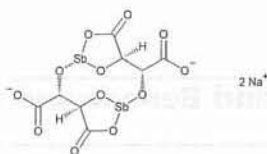
**Analysis:** Titrate the *Sample solution* with 0.010 N hydrochloric acid or 0.010 N sodium hydroxide to a pH of 4.5.

**Acceptance criteria:** NMT 2.0 mL

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## Antimony Sodium Tartrate



$C_8H_4Na_2O_{12}Sb_2$

581.61

Antimonate(2-), bis[μ-[2,3-dihydroxybutanedioato(4-)-O<sup>1</sup>,O<sup>2</sup>:O<sup>3</sup>,O<sup>4</sup>]]di-, disodium, stereoisomer;

Disodium bis[μ-[L-(+)-tartrato(4-)]diantimonate(2-)] [34521-09-0].

### DEFINITION

Antimony Sodium Tartrate contains NLT 98.0% and NMT 101.0% of antimony sodium tartrate ( $C_8H_4Na_2O_{12}Sb_2$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL,** *Antimony* (191), *Sodium* (191), and *Tartrate* (191)

### ASSAY

#### • PROCEDURE

**Sample:** 500 mg

**Analysis:** Dissolve the *Sample* in 50 mL of water, add 5 g of potassium sodium tartrate, 2 g of sodium borate,

and 3 mL of starch TS, and immediately titrate with 0.1 N iodine VS to the production of a persistent blue color. Each mL of 0.1 N iodine is equivalent to 14.54 mg of antimony sodium tartrate ( $C_8H_4Na_2O_{12}Sb_2$ ).  
Acceptance criteria: 98.0%–101.0% on the dried basis

### IMPURITIES

- **ARSENIC, Method II (211):** NMT 8 ppm

- **LEAD (251):** NMT 20 ppm

### SPECIFIC TESTS

#### • LOSS ON DRYING (731)

**Analysis:** Dry at 105° to constant weight.

**Acceptance criteria:** NMT 6.0%

#### • ACIDITY OR ALKALINITY

**Sample solution:** Dissolve 1.0 g in 50 mL of carbon dioxide-free water.

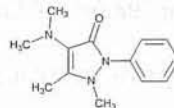
**Analysis:** Titrate the *Sample solution* with 0.010 N hydrochloric acid or 0.010 N sodium hydroxide to a pH of 4.5.

**Acceptance criteria:** NMT 2.0 mL

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## Antipyrine



$C_{11}H_{12}N_2O$

188.23

1,2-Dihydro-1,5-dimethyl-2-phenyl-3H-pyrazol-3-one;  
2,3-Dimethyl-1-phenyl-3-pyrazolin-5-one [60-80-0].

### DEFINITION

Antipyrine contains NLT 98.0% and NMT 102.0% of antipyrine ( $C_{11}H_{12}N_2O$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Solution A:** 0.77 g/L of ammonium acetate in water. Adjust with diluted ammonium hydroxide to a pH of 7.0 and pass through a filter of 0.2-µm pore size.

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
1.0	75	25
5.0	20	80
5.01	75	25
8.0	75	25

**System suitability solution:** 0.12 mg/mL of USP Antipyrine RS and 0.12 µg/mL of USP Antipyrine Related Compound A RS in water

**Standard solution:** 0.12 mg/mL of USP Antipyrine RS in water



**Sample solution:** 0.12 mg/mL of Antipyrine in water

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 2.1-mm × 10-cm; 1.8-μm packing L1

**Column temperature:** 35°

**Flow rate:** 0.4 mL/min

**Injection volume:** 1 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between antipyrine and antipyrine related compound A, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of antipyrine (C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O) in the portion of Antipyrine taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Antipyrine RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Antipyrine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** <281>: NMT 0.15%

**Delete the following:**

- **HEAVY METALS** <231>

**Test preparation:** Dissolve 1 g in 2 mL of 1 N acetic acid, and add water to make 25 mL.

**Acceptance criteria:** NMT 20 ppm (Official 1-Jan-2018)

- **ORGANIC IMPURITIES**

**Buffer:** Dissolve 6.8 g of monobasic potassium phosphate in 1 L of water, add 2 mL of triethylamine, and adjust with 5 N sodium hydroxide solution to a pH of 7.0.

**Mobile phase:** Methanol and *Buffer* (43:100)

**System suitability solution:** 5 μg/mL each of USP Antipyrine RS and USP Antipyrine Related Compound A RS in *Mobile phase*

**Standard solution:** 0.5 μg/mL of USP Antipyrine RS and 0.25 μg/mL of USP Antipyrine Related Compound A RS in *Mobile phase*

**Sample solution:** 500 μg/mL of Antipyrine in *Mobile phase*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 6.0-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**Run time:** 3 times the retention time of antipyrine

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 3.0 between antipyrine related compound A and antipyrine

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of antipyrine related compound A in the portion of Antipyrine taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of antipyrine related compound A from the *Sample solution*

$r_s$  = peak response of antipyrine related compound A from the *Standard solution*

$C_s$  = concentration of USP Antipyrine Related Compound A RS in the *Standard solution* (μg/mL)

$C_u$  = concentration of the *Sample solution* (μg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Antipyrine taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of any individual unspecified impurity from the *Sample solution*

$r_s$  = peak response of antipyrine from the *Standard solution*

$C_s$  = concentration of USP Antipyrine RS in the *Standard solution* (μg/mL)

$C_u$  = concentration of the *Sample solution* (μg/mL)

**Acceptance criteria:** See *Table 2*. Disregard any impurity peak less than 0.03%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Antipyrine related compound A	0.8	0.05
Antipyrine	1.0	—
Individual unspecified impurity	—	0.05
Total impurities	—	0.1

**SPECIFIC TESTS**

- **LOSS ON DRYING** <731>

**Analysis:** Dry at 60° for 2 h.

**Acceptance criteria:** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS** <11>

USP Antipyrine RS

USP Antipyrine Related Compound A RS

3-Methyl-1-phenyl-1H-pyrazol-5(4H)-one.

C<sub>10</sub>H<sub>10</sub>N<sub>2</sub>O 174.20

## Antipyrine and Benzocaine Otic Solution

» Antipyrine and Benzocaine Otic Solution is a solution of Antipyrine and Benzocaine in Glycerin. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of antipyrine (C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O) and benzocaine (C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub>). [NOTE—In the preparation of this Otic Solution, use Glycerin that has a low water content, in order that the Otic Solution may comply with the *Water* limit. This may be ensured by using Glycerin having a specific gravity of not less than 1.2607, corresponding to a concentration of 99.5 percent.]

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** <11>—

USP Antipyrine RS

USP Benzocaine RS



**Identification—**

A: Transfer 5 mL to a separator containing 25 mL of water, and extract the solution with two 25-mL portions of a mixture of equal volumes of ether and solvent hexane. Combine the extracts, and retain the water solution for *Identification* test B. Extract the ether-hexane solution with 50 mL of water, and discard the water layer. Evaporate the ether-hexane solution to dryness, dry the residue in vacuum at 40° to 50° for 1 hour, and dissolve the residue in 1 mL of chloroform: the IR absorption spectrum of this solution exhibits maxima at the same wavelengths as that of a similar solution of USP Benzocaine RS, concomitantly measured.

B: Add 5 mL of 1 N sodium hydroxide solution to the water solution retained from *Identification* test A, and extract with two 25-mL portions of chloroform. Evaporate the combined extracts to dryness, dry the residue in vacuum at 40° to 50° for 1 hour, and dissolve the residue in 3 mL of chloroform: the IR absorption spectrum of this solution exhibits maxima at the same wavelengths as that of a similar solution of USP Antipyrine RS, concomitantly measured.

C: The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water Determination, Method I** (921): not more than 1.0% is found.

**Assay—**

*Ammonium acetate solution*—Dissolve 7.7 g of ammonium acetate in water, dilute with water to 1000 mL, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of *Ammonium acetate solution* and acetonitrile (3:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Transfer about 15 mg of USP Benzocaine RS, accurately weighed, to a 100-mL volumetric flask, add 15/ *J* mg of USP Antipyrine RS, accurately weighed, *J* being the ratio of the labeled amount, in mg, of antipyrine to the labeled amount, in mg, of benzocaine per mL of Otic Solution. Add 50 mL of methanol, dissolve in methanol, dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Assay preparation*—Transfer an accurately measured volume of Otic Solution, equivalent to about 15 mg of benzocaine, to a 100-mL volumetric flask. Dissolve in 50 mL of methanol, dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 15-cm column that contains 5-μm packing L15. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor for the benzocaine peak is not more than 2.5, the column efficiency for the benzocaine peaks is not less than 1500 theoretical plates, and the relative standard deviation for replicate injections is not more than 2%.

*Procedure*—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.35 for antipyrine, and 1.0 for benzocaine. Calculate the quantity, in mg, of benzocaine (C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub>) in each mL of the Otic Solution taken by the formula:

$$1000(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Benzocaine RS in the *Standard preparation*; *V* is the volume, in mL, of Otic Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses due to benzocaine in the *Assay preparation* and the

*Standard preparation*, respectively. Calculate the quantity, in mg, of antipyrine (C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O) in each mL of the Otic Solution taken by the same formula, changing the terms to refer to antipyrine instead of benzocaine.

## Antipyrine, Benzocaine, and Phenylephrine Hydrochloride Otic Solution

» Antipyrine, Benzocaine, and Phenylephrine Hydrochloride Otic Solution is a solution of Antipyrine, Benzocaine, and Phenylephrine Hydrochloride in a suitable nonaqueous solvent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of antipyrine (C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O), benzocaine (C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub>), and phenylephrine hydrochloride (C<sub>9</sub>H<sub>13</sub>NO<sub>2</sub> · HCl).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Antipyrine RS

USP Benzocaine RS

USP Phenylephrine Hydrochloride RS

**Identification**—The retention times of the major peaks in the chromatograms of the *Assay preparations* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Assay—**

*Mobile phase*—Mix 480 mL of acetonitrile, 3520 mL of a 0.005 M solution of sodium 1-heptanesulfonate in water, and 4 mL of phosphoric acid.

*Standard preparation*—Accurately weigh about 25 mg of USP Antipyrine RS, about 25 mg of USP Benzocaine RS, and about 25 mg of USP Phenylephrine Hydrochloride RS into a 250-mL volumetric flask. Add 5 mL of a 0.5 mg per mL solution of *p*-aminobenzoic acid in *Mobile phase*. Add 150 mL of *Mobile phase*, and mix to effect solution, sonicating if necessary. Dilute with *Mobile phase* to volume, and mix.

*Assay preparation A*—Transfer an accurately measured volume of Otic Solution, equivalent to about 100 mg of antipyrine, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Assay preparation B*—Transfer an accurately measured volume of Otic Solution, equivalent to about 100 mg of benzocaine, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Assay preparation P*—Transfer an accurately measured volume of Otic Solution, equivalent to about 5 mg of phenylephrine hydrochloride, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 272-nm detector and a 4.6-mm × 30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.19 for *p*-aminobenzoic acid, 0.26 for phenylephrine, 0.64 for antipyrine, and 1.0 for benzocaine; the resolution, *R*, between phenylephrine and aminobenzoic acid is not less than 1.5, and the relative standard deviation for replicate injections is not more than 3.0%.



**Procedure**—Separately inject equal volumes (about 20 or 25  $\mu$ L) of the *Standard preparation* and each of the *Assay preparations* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of antipyrine ( $C_{11}H_{12}N_2O$ ) in each mL of the Otic Solution taken by the formula:

$$(C/V)(r_U/r_S)$$

in which *C* is the concentration, in  $\mu$ g per mL of USP Antipyrine RS in the *Standard preparation*; *V* is the volume, in mL, of Otic Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the antipyrine peak responses obtained from *Assay preparation A* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of benzocaine ( $C_9H_{11}NO_2$ ) in each mL of the Otic Solution taken by the formula:

$$(C/V)(r_U/r_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP Benzocaine RS in the *Standard preparation*; *V* is the volume, in mL, of Otic Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the benzocaine peak responses obtained from *Assay preparation B* and the *Standard preparation*, respectively. Calculate the quantity, in  $\mu$ g of phenylephrine hydrochloride ( $C_9H_{13}NO_2 \cdot HCl$ ) in each mL of the Otic Solution taken by the formula:

$$50(C/V)(r_U/r_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP Phenylephrine Hydrochloride RS in the *Standard preparation*; *V* is the volume, in mL, of Otic Solution taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the phenylephrine peak responses obtained from *Assay preparation P* and the *Standard preparation*, respectively.

## Antithrombin III Human

[9000-94-6].

### DEFINITION

Antithrombin III Human is a glycoprotein, which is the major inhibitor of thrombin and other activated clotting factors, including factors IX, X, XI, and XII. It is obtained from human plasma of healthy donors who have been tested and shown to be free from detectable agents of infection transmissible by transfusion of blood or blood derivatives. The manufacturing steps are shown to remove or inactivate known agents of infection. If substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to an acceptable level and that any residues are such as not to compromise the safety of the preparation for patients. When reconstituted in the recommended volume of diluent, the potency is NLT 25 International Units (IU)/mL. It contains 80%–120% of the potency stated on the label.

### IDENTIFICATION

- **A.** Meets the requirements of the *Assay*

### ASSAY

#### PROCEDURE

**Solution A:** Dissolve tris(hydroxymethyl)aminomethane, edetic acid, and sodium chloride in water to obtain a solution having concentrations of 0.050, 0.0075, and 0.175 M, respectively. Adjust with hydrochloric acid or sodium hydroxide solution to a pH of 8.4.

**Solution B:** 0.05% (w/v) of albumin human in *Solution A*

**Solution C:** 10 mg/mL of polybrene in *Solution B*

**Solution D:** Reconstitute thrombin bovine (factor IIa), and dilute with *Solution B* to obtain a solution having a concentration of 4 Thrombin IU/mL.

**Solution E:** Prepare a solution of chromogenic substrate for amidolytic test (see *Reagents, Indicators, and Solutions—Reagent Specifications*) for thrombin (factor IIa) in *Solution C* to obtain a solution having a concentration of about 40.0 mM.

**Solution F:** Resuspend USP Heparin Sodium for Assays RS according to the USP Certificate and dilute to 3 USP Heparin Units/mL in *Solution A*.

**Standard solutions:** Prepare seven dilutions from USP Antithrombin III Human RS within the linear range of the assay in *Solution F* (for example, 1.7, 1.5, 1.2, 1.0, 0.8, 0.6, and 0.4 IU/mL).

**Sample solutions:** Prepare three or more dilutions in *Solution F* within the linear range of the assay.

**Blank:** *Solution A*

**Analysis:** [NOTE—The procedure also can be performed using alternative platforms.]

For each dilution of the *Standard solution* and *Sample solution*, at least duplicates should be tested. Label a suitable number of tubes depending on the number of replicates that will be tested. For example, if five blanks will be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, and T3 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, S3, S4, S5, S6, and S7 each at least in duplicate for the dilutions of the *Standard solutions*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, S4, S5, S6, S7, B2, T1, T2, T3, B3, T1, T2, T3, B4, S1, S2, S3, S4, S5, S6, S7, B5.]

Prewarm *Solution D* and *Solution E* at 37°. Pipet 50  $\mu$ L each of the *Standard solutions*, *Sample solutions*, and *Blank* into suitable tubes placed in a water bath set at 37°. Add 350  $\mu$ L of prewarmed *Solution D* to each tube, mix, and incubate for 1 min. Add 100  $\mu$ L of prewarmed *Solution E* to each tube in the same order and mix. Follow the change in absorbance for each solution over 1 min at 405 nm using a suitable spectrophotometer (see *Ultraviolet-Visible Spectroscopy* (857)). Calculate the change in absorbance/min. Plot standard concentrations against resulting absorbance values and determine potency by interpolating from the standard curve using mean sample absorbances.

#### System suitability

**Samples:** *Standard solutions* and *Sample solutions*

The *R<sup>2</sup>* value of the standard curve is NLT 0.99. The initial and final blanks differ by NMT 10%. The absorbances of the three dilutions of the *Sample solution* must fall within the range of absorbances of the standard curve. The three dilutions of the *Sample solution* give potency estimates that differ by NMT 10%.

**Acceptance criteria:** 80%–120% of the potency stated on the label.

### IMPURITIES

#### HEPARIN CONTENT

**Solution A:** 9 g/L of sodium chloride

**Sheep plasma substrate:** Use sheep plasma suitable for the test procedure. If frozen, thaw at 37°.

**APTT reagent:** Use a suitable activated partial thromboplastin time (APTT) reagent containing phospholipid and a contact activator at a dilution giving a suitable blank recalcification time not exceeding 60 s.

**Calcium chloride solution:** 3.7 g/L of calcium chloride

**System suitability solution:** 5 IU/mL of heparin sodium for assays in USP Antithrombin III Human RS

**Standard solutions:** Make three or more dilutions of USP Heparin Sodium for Assays RS to known concentrations in USP Heparin Units/mL that are in the expected



range of the sample (for example, 0.5–1.5 USP Heparin Units/mL).

**Sample solutions:** Make three or more dilutions of Antithrombin III Human in the range of the *Standard solution* dilutions.

**Blank:** *Solution A*

**Analysis:** [NOTE—The procedure also can be performed using alternative platforms.]

For each *System suitability solution*, *Standard solution*, and *Sample solution*, at least duplicates should be tested. Label a suitable number of tubes depending on the number of replicates that will be tested. For example, if five blanks will be used: B1, B2, B3, B4, and B5 for the blanks; T1, T2, and T3 each at least in duplicate for the dilutions of the *Sample solutions*; and S1, S2, and S3 each at least in duplicate for the dilutions of the *Standard solutions* and SS for the *System suitability solution*. Distribute the blanks over the series in such a way that they accurately represent the behavior of the reagents during the experiments. [NOTE—Treat the tubes in the order B1, S1, S2, S3, B2, SS, T1, T2, T3, B3, T1, T2, T3, SS, B4, S1, S2, S3, B5.] In the following order add 1.0 mL of thawed *Sheep plasma substrate* to 1.0 mL of the *Standard solution* dilutions or the *Sample solution* dilutions or the *System suitability solution*. After each addition, mix but do not allow bubbles to form. Transfer each tube to a water bath at 37°, allow to equilibrate at 37° for about 15 min, and add to each tube 1 mL of *APTT reagent* previously heated to 37°. After an appropriate time for the *APTT reagent* used, usually 2–5 min, add 1 mL of *Calcium chloride solution* previously heated to 37° and determine the clotting time. Plot standard concentrations against resulting clotting times and determine heparin content by interpolating from the standard curve using mean sample clotting times. For samples with clotting times longer than the lowest standard dilution, report the result as NMT the lowest *Standard solution* concentration.

#### System suitability

**Samples:** *Standard solutions* and *Sample solutions*  
The  $R^2$  value of the standard curve is NLT 0.99. The three dilutions of the *Sample solution* give heparin content estimates that differ by NMT 10%. The heparin content in the *System suitability solution* is in the range of 4.0–7.5 IU/mL.

**Acceptance criteria:** NMT 0.1 USP Heparin Unit/Antithrombin III IU

#### SPECIFIC TESTS

- **STERILITY TESTS** (71), *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*: Meets the requirements
- **WATER DETERMINATION** (921), *Method I*: NMT 3.0%
- **PYROGEN TEST** (151): Inject 50 USP Antithrombin III Units per kg of the rabbit's weight, calculated from the activity stated on the label: meets the requirements.
- **GENERAL SAFETY**: Meets the requirements for biologics in *Biological Reactivity Tests, In Vivo* (88), *Safety Tests—Biologicals*
- **OSMOLALITY AND OSMOLARITY** (785), *Osmolality*: Reconstitute with the diluent according to the manufacturer's instruction: NLT 240 mOsmol/kg for the solution.
- **pH** (791): Reconstitute with the diluent according to the manufacturer's instruction: 6.0–7.5.
- **MOLECULAR WEIGHT DISTRIBUTION**

**Mobile phase:** 0.05 M sodium phosphate (dibasic), 0.05 M sodium phosphate (monobasic), 0.4 M arginine monohydrochloride, and 0.05% sodium azide. Adjust with 1 N sodium hydroxide to a pH of 6. Degas and filter.

**Solution A:** 4–5 mg/mL of thyroglobulin in *Mobile phase*

**Sample solution:** 8–10 mg/mL of Antithrombin III Human

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Columns**

**Guard:** 7.5 mm × 7.5 cm guard column containing packing L59

**Analytical:** 7.5 mm × 30 cm analytical column containing packing L59

#### Temperatures

**Autosampler:** 7°

**Column:** Ambient

**Flow rate:** 0.5 mL/min maintained constant to ±1%

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Sample solution*

#### Suitability requirements

**Column efficiency:** NLT 2000 theoretical plates

**Tailing factor:** 0.9–1.3

#### Analysis

**Samples:** *Solution A* and *Sample solution*

**Acceptance criteria:** Note the retention times of the major peak in the *Solution A* chromatogram. The relative peak area of the high-molecular weight peak eluting at about the same retention time as the major peak in the *Solution A* chromatogram, or earlier, is NMT 13%.

#### • TOTAL PROTEIN CONTENT

**Solution A:** 1000 mg/mL of trichloroacetic acid in water

**Sample solution:** 7.5 mg/mL of Antithrombin III Human in 0.15 M sodium chloride solution

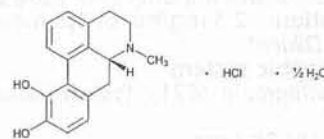
**Blank:** 0.15 M solution of sodium chloride

**Analysis:** To each of 2.0 mL of the *Sample solution* and the *Blank* in suitable centrifuge tubes, add 1.5 mL of *Solution A*. Mix, allow to stand for at least 10 min, centrifuge for 5 min, and decant the supernatant. Resuspend the precipitates in 1.5 mL of *Solution A*, centrifuge for 5 min, decant the supernatant, and hold the tubes inverted on a filter paper to drain. Quantitatively transfer the residues with a minimum quantity of water to a micro-Kjeldahl flask, and determine the nitrogen content (see *Nitrogen Determination* (461), *Method II*). Multiply the result, corrected for the *Blank*, by 6.25 to calculate the quantity of protein.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Use a Type I glass container with an appropriate stopper and seal. Store protected from light between 2° and 8°, excursions permitted up to 25°.
- **LABELING:** The labeling should state the content of antithrombin III in USP Antithrombin III Units. The diluent and the volume to be used to reconstitute the preparation are indicated.
- **USP REFERENCE STANDARDS** (11)  
USP Antithrombin III Human RS  
USP Heparin Sodium for Assays RS

### Apomorphine Hydrochloride



$\text{C}_{17}\text{H}_{17}\text{NO}_2 \cdot \text{HCl} \cdot \frac{1}{2}\text{H}_2\text{O}$

312.79

$\text{C}_{17}\text{H}_{17}\text{NO}_2 \cdot \text{HCl}$

303.79



4*H*-Dibenzo[*de,g*]quinoline-10,11-diol, 5,6,6a,7-tetrahydro-6-methyl-, hydrochloride, hemihydrate, (*R*)-;  
6a $\beta$ -Apomorphine-10,11-diol hydrochloride hemihydrate  
[41372-20-7].  
Anhydrous [314-19-2].

**DEFINITION**

Apomorphine Hydrochloride contains NLT 98.5% and NMT 101.5% of apomorphine hydrochloride ( $C_{17}H_{17}NO_2 \cdot HCl$ ), calculated on the dried basis.

**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197K)• **B. IDENTIFICATION TESTS—GENERAL**, Chloride (191)

**Sample solution:** 10 mg/mL of Apomorphine Hydrochloride in carbon dioxide-free water

**Analysis:** To 2 mL of the *Sample solution* add 0.1 mL of nitric acid. Mix, filter, and use the filtrate.

**Acceptance criteria:** Meets the requirements

**ASSAY**• **PROCEDURE**

**Sample solution:** Dissolve 250 mg of Apomorphine Hydrochloride in a mixture of 5.0 mL of 0.01 N hydrochloric acid and 50 mL of alcohol.

**Analysis:** Titrate the *Sample solution* with 0.1 N sodium hydroxide VS. Read the volume added between the first two points of inflexion. Each mL of 0.1 N sodium hydroxide is equivalent to 30.38 mg of apomorphine hydrochloride ( $C_{17}H_{17}NO_2 \cdot HCl$ ).

**Acceptance criteria:** 98.5%–101.5% on the dried basis

**IMPURITIES**• **RESIDUE ON IGNITION** (281): NMT 0.1%• **ORGANIC IMPURITIES**

**Diluent:** Glacial acetic acid and water (1:99)

**Solution A:** 1.1-g/L solution of sodium octanesulfonate, adjusted with diluted phosphoric acid (1:1) to a pH of 2.2

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	85	15
2	85	15
32	68	32
37	68	32

Return to original conditions and re-equilibrate the system.

**System suitability solution:** 0.25 mg/mL each of USP Apomorphine Hydrochloride RS and boldine in *Diluent*.

[NOTE—Boldine is 2,9-dihydroxy-1,10-dimethoxyapomorphine.]

**Standard solution:** 2.5  $\mu$ g/mL of USP Apomorphine Hydrochloride RS in *Diluent*

**Sensitivity solution:** 0.14  $\mu$ g/mL of USP Apomorphine Hydrochloride RS in *Diluent* from the *Standard solution*. [NOTE—The peak response of this solution is equivalent to that of a solution containing 1.25  $\mu$ g/mL of morphine hydrochloride, taking into account the relative response factor of this impurity (see Table 2).]

**Sample solution:** 2.5 mg/mL of Apomorphine Hydrochloride in *Diluent*

**Chromatographic system**

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m end-capped packing  
L1

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Sensitivity solution*

[NOTE—The typical relative retention times for boldine and apomorphine are about 0.9 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.5 between boldine and apomorphine, *System suitability solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Apomorphine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of each impurity from the *Sample solution*

$r_s$  = peak response of apomorphine from the *Standard solution*

$C_s$  = concentration of USP Apomorphine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Apomorphine Hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see Table 2)

**Acceptance criteria:** See Table 2. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Morphine	0.4	0.11	0.15
Apomorphine	1.0	—	—
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.5

**SPECIFIC TESTS**• **OPTICAL ROTATION**, *Specific Rotation* (781S)

**Sample solution:** 10 mg/mL in *Diluent*

**Diluent:** 2.06-g/L solution of hydrochloric acid in water

**Acceptance criteria:**  $-48^\circ$  to  $-52^\circ$ , determined at 20°

• **LOSS ON DRYING** (731)

**Analysis:** Dry a sample at 105° for 2 h.

**Acceptance criteria:** 2.0%–4.2%

• **COLOR OF SOLUTION**

**Sample solution:** Place 100 mg of Apomorphine Hydrochloride in a suitable test tube, add 10 mL of cold, oxygen-free water, and agitate gently until dissolved.

**Standard solution:** Dissolve 5 mg of Apomorphine Hydrochloride in 100.0 mL of water. Transfer 1.0 mL of this solution to a test tube of the same size as that used for the *Sample solution*. Dilute with 6 mL of water, add 1 mL of a 50-mg/mL sodium bicarbonate solution, and then add 0.50 mL of iodine TS. Allow to stand for 30 s, add 0.60 mL of a 25-mg/mL sodium thiosulfate solution, and dilute with water to 10 mL.

**Acceptance criteria:** The color of the *Sample solution*, observed promptly after the Apomorphine Hydrochloride has dissolved, is not more intense than that of a color of the *Standard solution*.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.



- **USP REFERENCE STANDARDS** (11)  
USP Apomorphine Hydrochloride RS

## Apomorphine Hydrochloride Tablets

» Apomorphine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{17}H_{17}NO_2 \cdot HCl \cdot \frac{1}{2}H_2O$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Apomorphine Hydrochloride RS

**Color of solution**—Dissolve a quantity of powdered Tablets, equivalent to 5 mg of apomorphine hydrochloride, in water to make 100.0 mL. Transfer 1.0 mL of the solution to a test tube, dilute with 6 mL of water, and, if necessary, filter through a small pledget of cotton. Add 1 mL of sodium bicarbonate solution (1 in 20), then add 0.50 mL of iodine TS. Allow to stand for 30 seconds, then add 0.60 mL of sodium thiosulfate solution (1 in 40), and dilute with water to 10 mL. This solution represents the color standard.

Place a quantity of powdered Tablets, equivalent to about 50 mg of apomorphine hydrochloride, in a test tube of suitably small size, add 10.0 mL of cold, oxygen-free water, insert the stopper in the test tube, and agitate gently until no more dissolves; if necessary, filter immediately through a small pledget of cotton. The color of the solution, observed promptly after preparation, is not more intense than that of the color standard. Use closely matched test tubes for the comparison.

**Identification**—To 5 mL of a filtered solution of Tablets, containing about 10 mg of apomorphine hydrochloride, add a slight excess of sodium bicarbonate solution (1 in 20): a white or greenish-white precipitate is formed. Add 3 drops of iodine TS, and shake vigorously: an emerald-green color is produced. Add 5 mL of ether, and, after vigorous shaking, allow the layers to separate: the ether is colored deep ruby-red while the water layer retains its green color.

**Disintegration** (701): 15 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Place 1 Tablet in a 500-mL volumetric flask containing 100 mL of 0.1 N hydrochloric acid, and shake for 15 minutes. Dilute with 0.1 N hydrochloric acid to volume, mix, and filter, discarding the first 20 mL of filtrate. Dilute a portion of the subsequent filtrate quantitatively and stepwise, if necessary, with 0.1 N hydrochloric acid to provide a solution containing approximately 12  $\mu$ g of apomorphine hydrochloride per mL. Concomitantly determine the absorbances of this solution and of a solution of USP Apomorphine Hydrochloride RS in the same medium having a known concentration of about 12  $\mu$ g of anhydrous apomorphine hydrochloride per mL, in 1-cm cells at the wavelength of maximum absorbance at about 273 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_{17}H_{17}NO_2 \cdot HCl \cdot \frac{1}{2}H_2O$  in the Tablet taken by the formula:

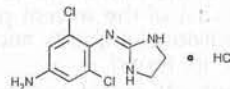
$$(312.80 / 303.79)(TC / D)(A_U / A_S)$$

in which 312.80 and 303.79 are the molecular weights of apomorphine hydrochloride hemihydrate and anhydrous apomorphine hydrochloride, respectively;  $T$  is the labeled quantity, in mg, of apomorphine hydrochloride in the Tablet;  $C$  is the concentration, in  $\mu$ g per mL, of anhydrous apo-

morphine hydrochloride in the Standard solution;  $D$  is the concentration, in  $\mu$ g per mL, of apomorphine hydrochloride in the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablet and the Standard solution, respectively.

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Dissolve an accurately weighed portion of the powder, equivalent to about 50 mg of apomorphine hydrochloride, in 25 mL of water in a separator, add 500 mg of sodium bicarbonate, and completely extract with successive small portions of ether. Combine the ether extracts in a separator, and wash them with three 5-mL portions of water. Shake the combined water washings with 10 mL of ether, and add this ether to the combined ether extracts. Extract the ether solutions with 20.0 mL of 0.02 N sulfuric acid VS, and wash with three 5-mL portions of water. Combine the acid extract and washings in a beaker, and warm on a steam bath to expel any residual ether. Cool, add methyl red TS, and titrate the excess acid with 0.02 N sodium hydroxide VS (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.02 N sulfuric acid is equivalent to 6.256 mg of  $C_{17}H_{17}NO_2 \cdot HCl \cdot \frac{1}{2}H_2O$ .

## Apraclonidine Hydrochloride



$C_9H_{10}Cl_2N_4 \cdot HCl$  281.57

1,4-Benzenediamine, 2,6-dichloro-N'-2-imidazolidinylidene-, monohydrochloride.

2-[(4-Amino-2,6-dichlorophenyl)imino]imidazolidine monohydrochloride [73218-79-8].

» Apraclonidine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_9H_{10}Cl_2N_4 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Apraclonidine Hydrochloride RS

**Identification**—

A: *Infrared Absorption* (197K).

B: It responds to the tests for *Chloride* (191).

**pH** (791): between 5.0 and 6.6 in a solution (1 in 100).

**Loss on drying** (731)—Dry it in vacuum at 105° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Delete the following:**

• **Heavy metals, Method II** (231): 0.002%. • (Official 1-Jan-2018)

**Chromatographic purity**—

**Phosphate buffer**—Transfer 6.8 mL of phosphoric acid to a 2000-mL volumetric flask, add about 1900 mL of water, and mix. Adjust with sodium hydroxide solution (1 in 2) to a pH of 3.0, dilute with water to volume, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, *Phosphate buffer*, and methanol (56:40:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).



**System suitability solution**—Prepare a solution in *Mobile phase* containing about 0.8 mg of USP Apraclonidine Hydrochloride RS per mL.

**Test solution**—Transfer about 20 mg of Apraclonidine Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and an 8-mm × 100-mm column that contains packing L7. The flow rate is about 3 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the tailing factor for the apraclonidine peak is not more than 2.2, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Inject about 20 µL of the *Test solution* into the chromatograph, record the chromatogram, and measure the areas for the major peaks. [NOTE—Allow about five times the elution time of apraclonidine before making the next injection.] Calculate the percentage of each peak, other than the solvent peak and the apraclonidine peak, in the specimen of Apraclonidine Hydrochloride taken by the same formula:

$$100r_i / r_t$$

in which  $r_i$  is the response of each peak other than the principal peak, and  $r_t$  is the sum of the responses of all of the peaks, excluding that of the solvent peak: not more than 1.0% for any individual impurity and not more than 2.0% total impurities are found.

**Assay**—Dissolve about 125 mg of Apraclonidine Hydrochloride, accurately weighed, in 40 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically from the second inflection point, using a calomel-glass electrode system (see *Titrimetry* (541)). Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 14.08 mg of  $C_9H_{10}Cl_2N_4 \cdot HCl$ .

## Apraclonidine Ophthalmic Solution

» Apraclonidine Ophthalmic Solution is a sterile, aqueous solution of Apraclonidine Hydrochloride. It contains an amount of apraclonidine hydrochloride ( $C_9H_{10}Cl_2N_4 \cdot HCl$ ) equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of apraclonidine ( $C_9H_{10}Cl_2N_4$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Apraclonidine Hydrochloride RS

### Identification—

A: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the major peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: Apply 2 µL of Apraclonidine Ophthalmic Solution and 2 µL of a Standard solution of USP Apraclonidine Hydrochloride RS in methanol containing about 11.5 mg per mL to a suitable high performance thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.2-mm layer of chromatographic silica gel mixture, or equivalent. Allow the

applications to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (74:22:4) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light. [NOTE—The apraclonidine spot should appear as a blue spot.] Spray the plate with fluorescamine solution, prepared by dissolving about 25 mg of fluorescamine in 25 mL of acetone. [NOTE—Avoid prolonged or repeated breathing of the aerosol from the fluorescamine spray. Also avoid prolonged or repeated contact with skin. Fluorescamine solution should be sprayed only in a hood.] Examine the plate under normal light and long-wavelength UV light. [NOTE—The apraclonidine spot should appear as a yellow spot under normal light and as a white spot under long-wavelength UV light.] The  $R_f$  value and appearance of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.4 and 7.8.

### Assay—

**Phosphate buffer**—Prepare as directed in the test for *Chromatographic purity* under *Apraclonidine Hydrochloride*.

**Mobile phase**—Prepare a filtered and degassed mixture of *Phosphate buffer*, acetonitrile, and methanol (68:30:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Apraclonidine Hydrochloride RS in water, and dilute quantitatively, and stepwise if necessary, with water to obtain a *Stock standard solution* having a known concentration of about 0.23 mg per mL. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having a known concentration of about 11.5 µg of USP Apraclonidine Hydrochloride RS per mL (equivalent to about 10 µg of apraclonidine per mL).

**Resolution solution**—Transfer about 1 mL of propiophenone to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 3.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 1.0 mL of this solution and 5.0 mL of the *Stock standard solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 20 mg of apraclonidine, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.5 mL of this solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and an 8-mm × 100-mm column that contains packing L7. The flow rate is about 3 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for apraclonidine and 1.0 for propiophenone; the column efficiency determined from the analyte peak is not less than 1000 theoretical plates; the tailing factor for the analyte peak is not more than 2.2; the resolution,  $R$ , between the analyte and propiophenone peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in

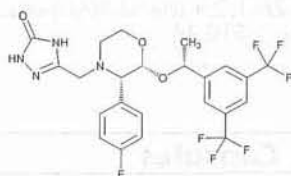


mg, of apraclonidine ( $C_9H_{10}Cl_2N_4$ ) in each mL of the Ophthalmic Solution taken by the formula:

$$(245.11 / 281.57)(2C / V)(r_U / r_S)$$

in which 245.11 and 281.57 are the molecular weights of apraclonidine and apraclonidine hydrochloride, respectively; C is the concentration, in  $\mu\text{g}$  per mL, of USP Apraclonidine Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Solution taken; and  $r_U$  and  $r_S$  are the apraclonidine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aprepitant



$C_{23}H_{21}F_7N_4O_3$

534.43

3H-1,2,4-Triazol-3-one, 5-[[[(2R,3S)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro-3-[[[(2R,3S)-3-(p-Fluorophenyl)-2-[( $\alpha$ R)- $\alpha$ -methyl-3,5-bis(trifluoromethyl)benzyl]oxy]morpholino]methyl]- $\Delta^2$ -1,2,4-triazolin-5-one; 3-[[[(2R,3S)-2-[(R)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)morpholino]methyl]-1H-1,2,4-triazol-5(4H)-one; 5-[[[(2R,3S)-2-[(R)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro-3H-1,2,4-triazol-3-one [170729-80-3].

### DEFINITION

Aprepitant contains NLT 98.0% and NMT 102.0% of aprepitant ( $C_{23}H_{21}F_7N_4O_3$ ), calculated on the anhydrous and solvent-free basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197)**  
[NOTE—Methods described under *Infrared Absorption* (197K), (197M), or (197A) may be used.]
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Dilute phosphoric acid:** Dilute 1 mL of phosphoric acid with water to 1 L.

**Mobile phase:** Acetonitrile and *Dilute phosphoric acid* (48:52)

**Diluent:** Acetonitrile and *Dilute phosphoric acid* (50:50)  
**Standard solution:** 0.2 mg/mL of USP Aprepitant RS in *Diluent*; sonicate as needed

**Sample solution:** 0.2 mg/mL of Aprepitant in *Diluent*; sonicate as needed

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu\text{m}$  packing L1

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu\text{L}$

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 0.73%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aprepitant ( $C_{23}H_{21}F_7N_4O_3$ ) in the portion of Aprepitant taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0%, on the anhydrous and solvent-free basis

### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

#### • ORGANIC IMPURITIES

**Dilute phosphoric acid and Diluent:** Proceed as directed in the *Assay*.

**Solution A:** *Dilute phosphoric acid*

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	58	42
25	58	42
45	30	70
50	30	70

Return to original conditions and re-equilibrate the system.

**System suitability solution:** 2.0 mg/mL of USP Aprepitant RS and 0.003 mg/mL of USP Desfluoro Aprepitant RS in *Diluent*, using sonication as necessary to dissolve

**Sensitivity solution:** 0.001 mg/mL of USP Aprepitant RS in *Diluent*

**Standard solution:** 0.003 mg/mL of USP Aprepitant RS in *Diluent*, using sonication as necessary to dissolve

**Sample solution:** 2.0 mg/mL of Aprepitant in *Diluent*, using sonication as necessary to dissolve

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu\text{m}$  packing L1

**Column temperature:** 35°

**Flow rate:** 1.0 mL/min

**Injection volume:** 10  $\mu\text{L}$

#### System suitability

**Samples:** *System suitability solution*, *Sensitivity solution*, and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between the desfluoro aprepitant and aprepitant peaks, *System suitability solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each individual impurity in the portion of Aprepitant taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response for aprepitant from the *Standard solution*

$C_S$  = concentration of USP Aprepitant RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aprepitant in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 2. Disregard any peak below 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desfluoro aprepitant	0.85	0.15
Aprepitant	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.30

• **LIMIT OF *S,R,S*-ENANTIOMER** (if present)

[NOTE—Perform this test if this impurity is possible from the manufacturing process.]

**Mobile phase:** Hexane and dehydrated alcohol (90:10)

**System suitability solution:** 0.08 mg/mL of USP Aprepitant RS and 0.08 mg/mL of USP Aprepitant Related Compound B RS in *Mobile phase*

**Sample solution:** 0.5 mg/mL of Aprepitant in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; packing L51

**Column temperature:** 30°

**Flow rate:** 0.5 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** Greater than 2.0 between the enantiomer peaks. [NOTE—The elution order is the *S,R,S*-enantiomer followed by the aprepitant peak, which is the *R,S,R*-enantiomer.]

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of the *S,R,S*-enantiomer in the portion of Aprepitant taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of the *S,R,S*-enantiomer

$r_T$  = sum of the peak responses of aprepitant and the *S,R,S*-enantiomer

**Acceptance criteria:** NMT 0.10% of the *S,R,S*-enantiomer

**SPECIFIC TESTS**

- **WATER DETERMINATION, Method Ia or Ic (921):** NMT 0.5%

• **OPTICAL ROTATION, Specific Rotation (781S)**

**Sample solution:** 10 mg/mL in methanol

**Acceptance criteria:** +66.0° to +71.0°

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Aprepitant RS

USP Aprepitant Related Compound B RS

*S,R,S*-Enantiomer: 3-[[[(2*S*,3*R*)-2-[(*S*)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)morpholino]methyl]-1*H*-1,2,4-triazol-5(4*H*)-one.

$C_{23}H_{21}F_7N_4O_3$  534.43

USP Desfluoro Aprepitant RS

5-[[[(2*R*,3*S*)-2-[(*R*)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-phenylmorpholino]methyl]-2*H*-1,2,4-triazol-3(4*H*)-one.

$C_{23}H_{22}F_6N_4O_3$  516.44

**Aprepitant Capsules****DEFINITION**

Aprepitant Capsules contain NLT 95.0% and NMT 105.0% of the labeled amount of aprepitant ( $C_{23}H_{21}F_7N_4O_3$ ).

**IDENTIFICATION**

• **A. ULTRAVIOLET ABSORPTION (197U)**

**Wavelength range:** 200–400 nm

**Standard solution:** 0.1 mg/mL of USP Aprepitant RS in methanol. Use sonication to dissolve.

**Sample solution:** Transfer the contents of Capsules, equivalent to 100 mg of aprepitant, to a 100-mL volumetric flask, add about 75 mL of methanol, and sonicate for about 5 min with intermittent shaking. Cool, dilute with methanol to volume, further dilute with methanol to obtain a solution containing 0.1 mg/mL of aprepitant, and pass through a nylon filter of 0.45-µm pore size.

**Acceptance criteria:** Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

• **PROCEDURE**

**Dilute phosphoric acid:** Dilute 1 mL of phosphoric acid with water to 1 L.

**Mobile phase:** Acetonitrile and *Dilute phosphoric acid* (45:55)

**Standard solution:** 0.05 mg/mL of USP Aprepitant RS in *Mobile phase*. Use sonication as necessary to dissolve.

**Sample solution:** Nominally 0.05 mg/mL of aprepitant in *Mobile phase*, prepared as follows. Mix the contents of NLT 20 Capsules, and transfer a portion of the contents, equivalent to 100 mg of aprepitant, to a 100-mL volumetric flask. Add about 75 mL of *Mobile phase* and sonicate for about 10 min with intermittent shaking. Cool, dilute to volume with *Mobile phase*, further dilute with *Mobile phase* to obtain a solution containing 0.05 mg/mL of aprepitant, and pass through a nylon filter of 0.45-µm pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aprepitant ( $C_{23}H_{21}F_7N_4O_3$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Aprepitant RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aprepitant in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Test 1

Medium: 2.2% Sodium dodecyl sulfate in water; 900 mL

Apparatus 2: 100 rpm, with sinkers. [NOTE—A suitable sinker is available from VanKel, www.chem.agilent.com, catalog number 12-3050. Proper placement of the Capsules is in the sinkers with the cap facing the fixed prong end.]

Time: 20 min

Dilute phosphoric acid: Dilute 1 mL of phosphoric acid with water to 1 L.

Mobile phase: Acetonitrile and *Dilute phosphoric acid* (50:50)

Standard solution: ( $L/900$ ) mg/mL of USP Aprepitant RS in *Medium*, where  $L$  is the label claim in mg/Capsule. Dissolve first in a minimal amount of methanol (using NMT 2% of the final volume) prior to diluting with *Medium*.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 50 μL for Capsules containing 40 mg/Capsule; 10 μL for all other strengths

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aprepitant ( $C_{23}H_{21}F_7N_4O_3$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Aprepitant RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Capsule)

Tolerances: NLT 80% ( $Q$ ) of the labeled amount of aprepitant ( $C_{23}H_{21}F_7N_4O_3$ ) is dissolved.

Test 2

Medium: 2.2% Sodium dodecyl sulfate in water; 900 mL

Apparatus 2: 100 rpm, with wire helix sinkers or other suitable sinkers

Time: 30 min

Dilute phosphoric acid and Mobile phase: Proceed as directed in the *Assay*.

Standard solution: ( $L/900$ ) mg/mL of USP Aprepitant RS in *Medium*, where  $L$  is the label claim in mg/Capsule. Dissolve first in a minimal amount of methanol (using NMT 2% of the final volume) prior to diluting with *Medium*.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.

Chromatographic system: Proceed as directed in the *Assay*, except use an autosampler temperature of 15°.

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aprepitant ( $C_{23}H_{21}F_7N_4O_3$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/L) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Aprepitant RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Capsule)

Tolerances: NLT 80% ( $Q$ ) of the labeled amount of aprepitant ( $C_{23}H_{21}F_7N_4O_3$ ) is dissolved.

• UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

Dilute phosphoric acid: Dilute 1 mL of phosphoric acid with water to 1 L.

Solution A: Acetonitrile and *Dilute phosphoric acid* (5:95)

Solution B: Acetonitrile and *Dilute phosphoric acid* (95:5)

Diluent: Acetonitrile and *Dilute phosphoric acid* (50:50)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
20	58	42
25	35	65
33	35	65

Return to original conditions and re-equilibrate the system for 10 min.

System suitability solution: 0.6 mg/mL of USP Aprepitant RS and 0.0012 mg/mL each of USP Desfluoro Aprepitant RS and USP Aprepitant Related Compound A RS in *Diluent*

Standard solution: 0.0012 mg/mL of USP Aprepitant RS in *Diluent*

Sample solution: Nominally 0.6 mg/mL of aprepitant, prepared as follows. Transfer the contents of Capsules, equivalent to 120 mg of aprepitant, to a 200-mL volumetric flask, add about 150 mL of *Diluent*, and sonicate for about 10 min with intermittent shaking. Cool, dilute



with Diluent to volume, and pass through a nylon filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

Column temperature: 35°

Flow rate: 1.0 mL/min

Injection volume: 10  $\mu$ L

#### System suitability

Samples: System suitability solution and Standard solution

#### Suitability requirements

Resolution: NLT 3.0 between the desfluoro aprepitant and aprepitant peaks, System suitability solution

Relative standard deviation: NMT 5.0%, Standard solution

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of any individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of each impurity from the Sample solution  
 $r_S$  = peak response of aprepitant from the Standard solution  
 $C_S$  = concentration of USP Aprepitant RS in the Standard solution (mg/mL)  
 $C_U$  = nominal concentration of aprepitant in the Sample solution (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desfluoro aprepitant	0.85	— <sup>a</sup>
Aprepitant	1.0	—
Aprepitant diastereomers (R,R,R and R,S,S) <sup>b</sup>	1.3	— <sup>a</sup>
Any other individual impurity	—	0.2
Total impurities	—	0.2

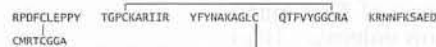
<sup>a</sup> Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

<sup>b</sup> The diastereomers are not separated by this procedure and should be identified based on the retention time of aprepitant related compound A (R,R,R-diastereomer), which is a component of the System suitability solution.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.
- **USP REFERENCE STANDARDS (11)**
  - USP Aprepitant RS
  - USP Aprepitant Related Compound A RS
  - R,R,R-Diastereomer: 3-[[[(2R,3R)-2-[(R)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)morpholino]methyl]-1H-1,2,4-triazol-5(4H)-one. C<sub>23</sub>H<sub>21</sub>F<sub>7</sub>N<sub>4</sub>O<sub>3</sub> 534.43
  - USP Desfluoro Aprepitant RS
  - S-[[[(2R,3S)-2-[(R)-1-[3,5-Bis(trifluoromethyl)phenyl]ethoxy]-3-phenylmorpholino]methyl]-2H-1,2,4-triazol-3(4H)-one. C<sub>23</sub>H<sub>22</sub>F<sub>6</sub>N<sub>4</sub>O<sub>3</sub> 516.44

## Aprotinin



C<sub>284</sub>H<sub>432</sub>N<sub>84</sub>O<sub>79</sub>S<sub>7</sub>

6511.44

Trypsin inhibitor, pancreatic basic;

L-Arginyl-L-prolyl-L-aspartyl-L-phenylalanyl-L-cysteinyll-L-leucyl-L-glutamyl-L-prolyl-L-prolyl-L-tyrosyl-L-threonylglycyl-L-prolyl-L-cysteinyll-L-lysyl-L-alanyl-L-arginyl-L-isoleucyl-L-isoleucyl-L-arginyl-L-tyrosyl-L-phenylalanyl-L-tyrosyl-L-asparaginyll-L-alanyl-L-lysyl-L-alanylglycyl-L-leucyl-L-cysteinyll-L-glutaminyll-L-threonyll-L-phenylalanyl-L-valyl-L-tyrosylglycylglycyl-L-cysteinyll-L-arginyl-L-alanyl-L-lysyl-L-arginyl-L-asparaginyll-L-asparaginyll-L-phenylalanyl-L-lysyl-L-seryl-L-alanyl-L-glutamyl-L-aspartyl-L-cysteinyll-L-methionyl-L-arginyl-L-threonyll-L-cysteinyllglycylglycyl-L-alanine cyclic (5→55), (14→38), (30→51) tris(disulfide) [9087-70-1].

#### DEFINITION

Aprotinin is a polypeptide consisting of a chain of 58 amino acid residues, which inhibits stoichiometrically the activity of several proteolytic enzymes such as chymotrypsin, kallikrein, plasmin, and trypsin. Aprotinin is obtained from bovine tissues and purified by a suitable process, and is stored as a bulk solution or lyophilized powder. Its potency, calculated on the dried basis, is NLT 3 USP Aprotinin Units/mg. In addition, the method of manufacture is validated to result in NMT 0.2  $\mu$ g of histamine per 3 USP Aprotinin Units using validated methods. The origin and sourcing of bovine material must be specified in compliance with FDA requirements. The manufacturing process is validated to demonstrate the clearance of potential infectious agents (i.e., viruses, TSE agents). One USP Aprotinin Unit is equivalent to 1800 Kallikrein Inhibition Units (K.I.U.).

#### IDENTIFICATION

##### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

Cupric chloride solution: 10 mg/mL of cupric chloride

Sample solution: A solution of Aprotinin in water containing about 15 USP Aprotinin Units/mL

##### Chromatographic system

Developing solvent system: To a mixture of glacial acetic acid and water (5:4) add 100 g/L of sodium acetate.

Spray reagent: Dissolve 0.1 g of ninhydrin in a mixture containing 6 mL of Cupric chloride solution, 21 mL of glacial acetic acid, and 70 mL of alcohol.

Analysis: Proceed as directed in the chapter, except spray the plate with the Spray reagent, and heat at 60° to visualize the spots.

- **B.** The retention time of the major peak of the Sample solution corresponds to that of the System suitability solution, as obtained in Limit of N-Pyroglutamyl-Aprotinin and Related Compounds.

#### ASSAY

##### • PROCEDURE

Buffer: Transfer about 0.93 g of boric acid into a 1000-mL volumetric flask, dissolve in 900 mL of water, adjust with 5 N sodium hydroxide to a pH of 8.0, and dilute with water to volume. Transfer 100 mL of this solution into a 1000-mL volumetric flask, and dilute with water to volume.

Sample solution: A solution of Aprotinin in Buffer containing about 1.67 USP Aprotinin Units/mL (about 0.6 mg/mL)

Trypsin solution: 4300 USP Trypsin Units/mL of USP Trypsin Crystallized RS in 0.001 N hydrochloric acid. Use a freshly prepared solution, and store in ice water.



**Trypsin and aprotinin solution:** To 4.0 mL of the *Trypsin solution* add 1.0 mL of the *Sample solution*. Dilute immediately with *Buffer* to 40.0 mL. Allow to stand at room temperature for 10 min, then keep in ice water. Use within 6 h of preparation.

**Dilute trypsin solution:** Dilute 0.5 mL of the *Trypsin solution* with *Buffer* to 10.0 mL. Allow to stand at room temperature for 10 min, then store in ice water.

**Substrate solution:** 6.9 mg/mL of *N*-benzoyl-L-arginine ethyl ester hydrochloride in water. Use within 2 h.

**Analysis:** Mix 9.0 mL of *Buffer* and 1.0 mL of *Substrate solution* in a jacketed glass vessel with a capacity of about 30 mL and containing a stirring device. The lid of the reaction vessel should contain five holes to accommodate the electrodes, the tip of a buret, a tube for the admission of nitrogen, and the introduction of reactants. An automated or manual titration apparatus may be used. Adjust with 0.1 N sodium hydroxide VS to a pH of 8.0. Maintain an atmosphere of nitrogen within the vessel, and stir continuously. When the temperature has reached equilibrium at  $25 \pm 0.1^\circ$ , add 1.0 mL of *Trypsin and aprotinin solution*, and start a timer. Maintain at a pH of 8.0 by the addition of 0.1 N sodium hydroxide VS, and record the volume added every 30 s. Continue the reaction for 6 min. Carry out a similar titration using 1.0 mL of the *Dilute trypsin solution*.

For the lyophilized powder, calculate the aprotinin activity in USP Aprotinin Units/mg:

$$\text{Result} = F_1 \times [(F_2 \times n_2 - n_1)/m]$$

$F_1$  = conversion factor, 4000

$F_2$  = difference in the amount of trypsin used in *Trypsin and aprotinin solution* and *Dilute trypsin solution*, 2

$n_2$  = volume of 0.1 N sodium hydroxide added per second, after adding *Dilute trypsin solution* (mL/s)

$n_1$  = volume of 0.1 N sodium hydroxide added per second, after adding *Trypsin and aprotinin solution* (mL/s)

$m$  = quantity of Aprotinin used to prepare 1 mL of the *Sample solution* (mg)

For the concentrated solution, calculate the USP Aprotinin Units/mL:

$$\text{Result} = F_1 \times (F_2 \times n_2 - n_1) \times D$$

$F_1$  = conversion factor, 4000

$F_2$  = difference in the amount of trypsin used in *Trypsin and aprotinin solution* and *Dilute trypsin solution*, 2

$n_2$  = volume of 0.1 N sodium hydroxide added per second, after adding *Dilute trypsin solution* (mL/s)

$n_1$  = volume of 0.1 N sodium hydroxide added per second, after adding *Trypsin and aprotinin solution* (mL/s)

$D$  = dilution factor of the concentrated solution used to prepare the *Sample solution*

**Acceptance criteria:** NLT 3 USP Aprotinin Units/mg on the dried basis

## IMPURITIES

### • LIMIT OF des-ALA-APROTIMIN AND des-ALA-des-GLY-APROTIMIN

**Buffer:** Dissolve 8.21 g of monobasic potassium phosphate in 400 mL of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 500 mL.

**System suitability solution:** Dilute USP Aprotinin RS with water to obtain a solution containing about 4–7 USP Aprotinin Units/mL.

**Sample solution:** Dilute a concentrated solution of Aprotinin with water, or weigh out Aprotinin and dis-

solve in water to obtain about 4–7 USP Aprotinin Units/mL of Aprotinin.

**Capillary rinse procedure:** Rinse the capillary for NLT 1 min with NLT 10 total capillary volumes of 0.1 N sodium hydroxide, followed by at least 10 total capillary volumes of water and by at least 20 capillary volumes of *Buffer* between injections.

### Electrophoretic system

**Mode:** CE

**Detector:** UV 214 nm

**Capillary:** 75- $\mu$ m  $\times$  45- to 60-cm uncoated fused silica. Use *Buffer* as the electrolyte in both buffer reservoirs.

**Capillary temperature:**  $25^\circ$

**Injection procedure:** Transfer a volume of the *Sample solution*, approximately 15 nL, into the anodic end of the capillary. Apply differential pressure of 3.5 kPa for 3 s either by vacuum or pressure.

**Applied voltage:** 0.2 kV/cm

**Run time:** 30 min

**System suitability:** The baseline is stable and shows little drift.

**Sample:** *System suitability solution*

### Suitability requirements

**Migration time:** 19–25 min for aprotinin

**Relative migration times:** About 0.98 for des-Ala-des-Gly-aprotinin, 0.99 for des-Ala-aprotinin, and 1 for aprotinin

**Resolution:** NLT 0.8 between the des-Ala-des-Gly-aprotinin and des-Ala-aprotinin peaks, and NLT 0.5 between the des-Ala-aprotinin and aprotinin peaks

**Tailing factor:** NMT 3 for aprotinin (see *Chromatography* (621) for calculation)

### Analysis

**Sample:** *Sample solution*

Calculate the percentage of des-Ala-des-Gly-aprotinin and des-Ala-aprotinin:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of des-Ala-des-Gly-aprotinin or des-Ala-aprotinin

$r_T$  = sum of the peak responses of des-Ala-des-Gly-aprotinin, des-Ala-aprotinin, and aprotinin

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Migration Time	Acceptance Criteria, NMT (%)
des-Ala-des-Gly- aprotinin	0.98	8.0
des-Ala-aprotinin	0.99	7.5
Aprotinin	1	—

### • LIMIT OF N-PYROGLUTAMYL-APROTIMIN AND RELATED COMPOUNDS

**Solution A:** 3.52 g/L of monobasic potassium phosphate and 7.26 g/L of dibasic sodium phosphate. Filter, and degas.

**Solution B:** 3.52 g/L of monobasic potassium phosphate, 7.26 g/L of dibasic sodium phosphate, and 66.07 g/L of ammonium sulfate. Filter, and degas.

**Mobile phase:** See *Table 2*.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	92	8
21	64	36
30	0	100



Table 2 (Continued)

Time (min)	Solution A (%)	Solution B (%)
31	92	8
40	92	8

**System suitability solution:** USP Aprotinin System Suitability RS in Solution A, with 5 USP Aprotinin Units/mL

**Sample solution:** Aprotinin in Solution A, with 5 USP Aprotinin Units/mL

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 7.5-mm × 7.5-cm; packing L52

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 40 µL

#### System suitability

**Sample:** System suitability solution

#### Suitability requirements

**Retention time:** 17–20 min for aprotinin

**Relative retention times:** 0.9 and 1.0 for N-pyroglutamyl-protinin and aprotinin, respectively

**Resolution:** NLT 1.0 between N-pyroglutamyl-protinin and aprotinin

**Tailing factor:** NMT 2.0 for aprotinin

#### Analysis

**Sample:** Sample solution

Calculate the percentage of each impurity peak:

$$\text{Result} = (r_u/r_T) \times 100$$

$r_u$  = peak response of each impurity

$r_T$  = sum of all the peak responses from the Sample solution

**Acceptance criteria:** See Table 3.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
N-Pyroglutamyl-protinin	0.9	1.0
Aprotinin	1.0	—
Any other impurity	—	0.5
Sum of all unknown impurities	—	1.0

#### • LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS

**Mobile phase:** Acetonitrile, glacial acetic acid, and water (1:1:3). Filter and degas.

**System suitability solution:** Aprotinin solution that contains about 5 USP Aprotinin Units/mL with about 2% aprotinin oligomers. [NOTE—This solution can be obtained by heating lyophilized aprotinin at 112° for about 2 h and dissolving the solid at the specified concentration in water.]

**Sample solution:** Aprotinin in water, with about 5 USP Aprotinin Units/mL

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Columns:** Series of three 7.8-mm × 30-cm columns; packing L33

**Flow rate:** 1.0 mL/min

**Injection volume:** 100 µL

#### System suitability

**Sample:** System suitability solution

#### Suitability requirements

**Retention time:** 24.5–25.5 min for aprotinin

**Relative retention times:** 0.9 and 1.0 for the dimer and aprotinin, respectively

**Resolution:** NLT 1.3 between the dimer and aprotinin peaks

**Tailing factor:** NMT 2.5 for aprotinin

#### Analysis

**Sample:** Sample solution

Calculate the percentage of each oligomer peak:

$$\text{Result} = (r_u/r_T) \times 100$$

$r_u$  = response of each peak with a retention time less than that of the aprotinin monomer

$r_T$  = sum of all the peak responses

**Acceptance criteria:** Sum of all the oligomer peak responses is NMT 1.0%.

#### SPECIFIC TESTS

##### • ABSORBANCE

(See Ultraviolet-Visible Spectroscopy (857).)

**Sample solution:** 3.0 USP Aprotinin Units/mL

**Acceptance criteria:** The Sample solution exhibits an absorption maximum at 277 nm. The absorbance at the maximum is NMT 0.80.

##### • BIOLOGICAL REACTIVITY TESTS, IN VIVO, Safety Tests—Biologicals (88)

**Sample solution:** Prepare a solution of Aprotinin that contains 4 USP Aprotinin Units/mL using a sufficient quantity of Water for Injection.

**Acceptance criteria:** Meets the requirements

##### • SPECIFIC ACTIVITY OF THE DRY RESIDUE

This test should only be performed when product is a concentrated solution.

**Sample solution:** 25.0 mL of Aprotinin concentrated solution

**Analysis:** Evaporate the Sample solution to dryness in a water bath, dry the residue at 110° for 15 h, and weigh. From the weight of the residue and the activity determined in the Assay, calculate the number of USP Aprotinin Units/mg of dry residue.

**Acceptance criteria:** NLT 3.0 USP Aprotinin Units/mg of dried residue

##### • LOSS ON DRYING (731)

This test should only be performed on the lyophilized powder.

**Sample:** 100 mg

**Analysis:** Dry the Sample in a capillary-stoppered bottle under vacuum at a pressure of NMT 5 mm of mercury at 60° for 3 h.

**Acceptance criteria:** NMT 6.0%

##### • BACTERIAL ENDOTOXINS TEST (85)

**Sample solution:** 6 USP Aprotinin Units/mL

**Acceptance criteria:** NMT 0.14 USP Endotoxin Unit per USP Aprotinin Unit

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** For lyophilized powder, preserve in tight containers, and store in a cold place. Protect from light. For bulk solution, preserve in tight containers at a temperature not exceeding 25°. Avoid freezing.

• **LABELING:** The labeling states the source of the material and the number of Kallikrein Inhibition Units/mg or the number of Kallikrein Inhibition Units/mL.



- **USP REFERENCE STANDARDS (11)**
  - USP Aprotinin RS
  - USP Aprotinin System Suitability RS
  - USP Endotoxin RS
  - USP Trypsin Crystallized RS

## Aprotinin Injection

### DEFINITION

Aprotinin Injection is a sterile solution of Aprotinin in Water for Injection that also contains sodium chloride. One USP Aprotinin Unit is equivalent to 1800 Kallikrein Inhibition Units (K.I.U.). It contains NLT 90.0% and NMT 110.0% of the potency stated on the label, expressed in K.I.U./mL.

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the test for *Limit of N-Pyroglutamyl-Aprotinin and Related Compounds*.
- **B.** It meets the requirements in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** Transfer about 0.93 g of boric acid into a 1000-mL volumetric flask, dissolve in 900 mL of water, adjust with 5 N sodium hydroxide to a pH of 8.0, and dilute with water to volume. Transfer 100 mL of this solution into a 1000-mL volumetric flask, and dilute with water to volume.

**Sample solution:** A solution of aprotinin in *Buffer* containing about 1.67 USP Aprotinin Units/mL (about 0.6 mg/mL).

**Trypsin solution:** 4300 USP Trypsin Units/mL of USP Trypsin Crystallized RS in 0.001 N hydrochloric acid. Use a freshly prepared solution, and store in ice water.

**Trypsin and aprotinin solution:** To 4.0 mL of the *Trypsin solution*, add 1.0 mL of the *Sample solution*. Dilute immediately with *Buffer* to 40.0 mL. Allow to stand at room temperature for 10 min, then keep in ice water. Use within 6 h of preparation.

**Dilute trypsin solution:** Dilute 0.5 mL of the *Trypsin solution* with *Buffer* to 10.0 mL. Allow to stand at room temperature for 10 min, then store in ice water.

**Substrate solution:** 6.9 mg/mL of *N*-benzoyl-L-arginine ethyl ester hydrochloride. Use within 2 h.

**Analysis:** Mix 9.0 mL of *Buffer* and 1.0 mL of *Substrate solution* in a jacketed-glass vessel with a capacity of about 30 mL and containing a stirring device. The lid of the reaction vessel should contain five holes to accommodate the electrodes, the tip of a buret, a tube for the admission of nitrogen, and the introduction of reactants. An automated or manual titration apparatus may be used. Adjust with 0.1 N sodium hydroxide VS to a pH of 8.0. Maintain an atmosphere of nitrogen within the vessel, and stir continuously. When the temperature has reached equilibrium at  $25 \pm 0.1^\circ$ , add 1.0 mL of *Trypsin and aprotinin solution*, and start a timer. Maintain at a pH of 8.0 by the addition of 0.1 N sodium hydroxide VS, and record the volume added every 30 s. Continue the reaction for 6 min. Carry out a similar titration using 1.0 mL of the *Dilute trypsin solution*. Calculate the potency in USP Aprotinin Units/mL:

$$\text{Result} = F_1 \times (F_2 \times n_2 - n_1) \times D$$

$F_1$  = conversion factor, 4000

$F_2$  = difference in the amount of trypsin used in *Trypsin and aprotinin solution* and *Dilute trypsin solution*, 2

$n_2$  = volume of 0.1 N sodium hydroxide added per second, after adding *Dilute trypsin solution* (mL/s)

$n_1$  = volume of 0.1 N sodium hydroxide added per second, after adding *Trypsin and aprotinin solution* (mL/s)

$D$  = dilution factor used to prepare the *Sample solution*

**Acceptance criteria:** 90.0%–110.0% of the potency stated on the label, expressed in K.I.U./mL

### OTHER COMPONENTS

#### CONTENT OF SODIUM CHLORIDE

**Sample:** 5.0 mL of Injection

**Analysis:** Pipet the *Sample* into a beaker containing 50 mL of water. Add 10 mL of 25% nitric acid. Titrate with 0.1 N silver nitrate VS to a potentiometric endpoint, using a silver combination electrode. Perform a blank determination (see *Titrimetry* (541)), and make any necessary correction. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride.

**Acceptance criteria:** 42.5–47.5 mg

### IMPURITIES

#### LIMIT OF N-PYROGLUTAMYL-APROTIMIN AND RELATED COMPOUNDS

**Solution A:** 3.52 g/L of monobasic potassium phosphate and 7.26 g/L of dibasic sodium phosphate. Filter, and degas.

**Solution B:** 3.52 g/L of monobasic potassium phosphate, 7.26 g/L of dibasic sodium phosphate, and 66.07 g/L of ammonium sulfate. Filter, and degas.

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	92	8
21	64	36
30	0	100
31	92	8
40	92	8

**System suitability solution:** USP Aprotinin System Suitability RS in *Solution A* with 5 USP Aprotinin Units/mL

**Sample solution:** Aprotinin in *Solution A*, with 5 USP Aprotinin Units/mL

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 7.5-mm × 7.5-cm; packing L52

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 40 µL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Retention time:** 17–20 min for aprotinin

**Relative retention times:** 0.9 and 1.0 for *N*-pyroglutamyl-aprotinin and aprotinin, respectively

**Resolution:** NLT 1.0 between *N*-pyroglutamyl-aprotinin and aprotinin

**Tailing factor:** NMT 2.0 for the aprotinin peak

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity peak:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity

$r_T$  = sum of all the peak responses from the *Sample solution*



Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
N-Pyroglutamyl- aprotinin	0.9	1.0
Aprotinin	1.0	—
Any other impurity	—	0.5
Sum of all unknown impurities	—	1.0

• **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**

**Mobile phase:** Acetonitrile, glacial acetic acid, and water (1:1:3). Filter, and degas.

**System suitability solution:** Aprotinin solution that contains about 5 USP Aprotinin Units/mL with about 2% aprotinin oligomers. [NOTE—This solution can be obtained by heating lyophilized aprotinin at 112° for about 2 h and dissolving the solid at the specified concentration in water.]

**Sample solution:** Aprotinin in water, with 5 USP Aprotinin Units/mL

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** Series of three 7.8-mm × 30-cm columns; packing L33

**Flow rate:** 1.0 mL/min

**Injection volume:** 100 μL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Retention time:** 24.5–25.5 min for aprotinin

**Relative retention times:** 0.9 and 1.0 for the dimer and aprotinin, respectively

**Resolution:** NLT 1.3 between the dimer and aprotinin peaks

**Tailing factor:** NMT 2.5 for the aprotinin peak

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each oligomer peak:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = response of each peak with a retention time less than that of the aprotinin monomer

$r_T$  = sum of all the peak responses

**Acceptance criteria:** Sum of all the oligomer peak responses is NMT 1.5%.

**SPECIFIC TESTS**

• **STERILITY TESTS** <71>: It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.

• **PARTICULATE MATTER IN INJECTIONS** <788>: Meets the requirements

• **PH** <791>: 4.5–6.5

• **INJECTIONS AND IMPLANTED DRUG PRODUCTS** <1>: Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** <85>: It contains NMT 0.14 USP Endotoxin Units per USP Aprotinin Unit.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in single-dose containers. Store at up to 25°, and avoid freezing.

• **USP REFERENCE STANDARDS** <11>

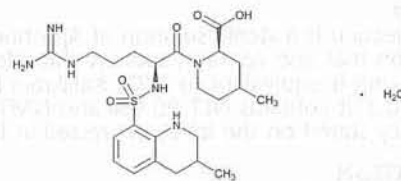
USP Aprotinin RS

USP Aprotinin System Suitability RS

USP Endotoxin RS

USP Trypsin Crystallized RS

## Argatroban



$C_{23}H_{36}N_6O_5S \cdot H_2O$  526.65

2-Piperidinecarboxylic acid, 1-[(S)-5-[(aminoiminomethyl)amino]-1-oxo-2-[[[1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]amino]pentyl]-4-methyl-, (2R,4R)-monohydrate;

(2R,4R)-4-Methyl-1-{N<sup>2</sup>-[(1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-L-arginyl}pipecolic acid, monohydrate [141396-28-3].

**DEFINITION**

Argatroban contains NLT 98.0% and NMT 102.0% of argatroban ( $C_{23}H_{36}N_6O_5S$ ), calculated on the anhydrous and solvent-free basis.

**IDENTIFICATION**

• **A. INFRARED ABSORPTION** <197K>

• **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

• **PROCEDURE**

[NOTE—It is recommended to keep all solutions containing argatroban at about 4°.]

**Solution A:** 10 mM ammonium acetate and 5 mM sodium 1-heptanesulfonate

**Solution B:** Acetonitrile and methanol (500:300)

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	60	40
20	60	40
35	50	50
50	20	80
60	20	80
60.1	60	40
72.1	60	40

**Standard solution:** 4 mg/mL of USP Argatroban RS in methanol

**Sample solution:** 4 mg/mL of Argatroban in methanol

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)



Mode: LC  
 Detector: UV 259 nm  
 Column: 4.6-mm × 25-cm; 3-μm packing L1  
 Temperatures  
 Column: 50°  
 Autosampler: 4°  
 Flow rate: 0.6 mL/min  
 Injection volume: 10 μL  
 System suitability  
 Sample: *Standard solution*  
 Suitability requirements  
 Tailing factor: NMT 1.5 for both peaks  
 Relative standard deviation: NMT 1.0% for the sum of the peak responses of (R)-argatroban and (S)-argatroban

#### Analysis

Samples: *Standard solution* and *Sample solution*  
 Calculate the percentage of argatroban (C<sub>23</sub>H<sub>36</sub>N<sub>6</sub>O<sub>5</sub>S) in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = sum of the peak responses of (R)-argatroban and (S)-argatroban from the *Sample solution*

$r_S$  = sum of the peak responses of (R)-argatroban and (S)-argatroban from the *Standard solution*

$C_S$  = concentration of USP Argatroban RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Argatroban in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous and solvent-free basis

#### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

#### • ORGANIC IMPURITIES

[NOTE—It is recommended to keep all solutions containing argatroban at about 4°.]

Mobile phase, *Sample solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.

Sensitivity solution: 2 μg/mL of USP Argatroban RS in methanol

Standard solution: 4 μg/mL each of USP Argatroban RS, USP Argatroban Related Compound A RS, and USP Argatroban Related Compound B RS in methanol

#### System suitability

Samples: *Sensitivity solution* and *Standard solution*

#### Suitability requirements

Resolution: NLT 1.2 between (R)-argatroban and (S)-argatroban, *Standard solution*

Signal-to-noise ratio: NLT 10 for (R)-argatroban, *Sensitivity solution*

Relative standard deviation: NMT 5% for all peaks. For argatroban, use the sum of the peak responses of (R)-argatroban and (S)-argatroban, *Standard solution*.

#### Analysis

Samples: *Standard solution* and *Sample solution*  
 Calculate the percentage of argatroban related compound A and argatroban related compound B in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of argatroban related compound A or argatroban related compound B from the *Sample solution*

$r_S$  = peak response of argatroban related compound A or argatroban related compound B from the *Standard solution*

$C_S$  = concentration of USP Argatroban Related Compound A RS or USP Argatroban Related Compound B RS in the *Standard solution*. (mg/mL)

$C_U$  = concentration of Argatroban in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity from the *Sample solution*

$r_S$  = sum of the peak responses of (R)-argatroban and (S)-argatroban from the *Standard solution*

$C_S$  = concentration of USP Argatroban RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Argatroban in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Argatroban related compound A <sup>a</sup>	0.23	0.15
Argatroban related compound B <sup>b</sup>	0.39	0.15
(R)-Argatroban <sup>c</sup>	1.00	—
(S)-Argatroban <sup>d</sup>	1.03	—
Any unspecified impurity	—	0.10
Total impurities <sup>e</sup>	—	0.5

<sup>a</sup> (2R,4R)-1-[N<sup>o</sup>-Nitro-N<sup>2</sup>-(3-methylquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.

<sup>b</sup> Ethyl (2R,4R)-1-[N<sup>o</sup>-nitro-L-arginyl]-4-methylpiperidine-2-carboxylate hydrochloride.

<sup>c</sup> (2R,4R)-4-Methyl-1-[N<sup>2</sup>-[[(R)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-L-arginyl]pipecolic acid.

<sup>d</sup> (2R,4R)-4-Methyl-1-[N<sup>2</sup>-[[(S)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-L-arginyl]pipecolic acid.

<sup>e</sup> Total impurities include specified and unspecified impurities and argatroban related compound C from the test for *Content of Argatroban Related Compound C*.

#### • CONTENT OF ARGATROBAN RELATED COMPOUND C

[NOTE—It is recommended to keep all solutions containing argatroban at about 4°.]

Buffer: 10 mM ammonium acetate and 5 mM sodium 1-heptanesulfonate

Solution A: Acetonitrile, dehydrated alcohol, and *Buffer* (80:240:680)

Mobile phase: See Table 3.

Table 3

Time (min)	Solution A (%)	Acetonitrile (%)
0	100	0
70	100	0
71	30	70
91	30	70
92	100	0
102	100	0

Sensitivity solution: 4 μg/mL of USP Argatroban Related Compound C RS in methanol

System suitability solution: 10 mg/mL of USP Argatroban RS and 0.1 mg/mL of USP Argatroban Related Compound C RS in methanol

Sample solution: 10 mg/mL of Argatroban in methanol  
 Chromatographic system: Proceed as directed in the *Assay*.



**System suitability**

**Samples:** Sensitivity solution and System suitability solution

**Suitability requirements**

**Resolution:** NLT 1.4 between argatroban related compound C and (R)-argatroban, *System suitability solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Relative standard deviation:** NMT 2.5% for argatroban related compound C, *System suitability solution*

**Analysis**

**Sample:** Sample solution

Calculate the percentage of argatroban related compound C in the portion of Argatroban taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of argatroban related compound C from the *Sample solution*

$r_T$  = total of all peak responses from the *Sample solution*

**Acceptance criteria:** See Table 4.

**Table 4**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Argatroban related compound C <sup>a</sup>	0.94	0.15
(R)-Argatroban	1.00	—
(S)-Argatroban	1.07	—

<sup>a</sup> (2R,4R)-1-[N<sup>8</sup>-Amino-N<sup>2</sup>-(3-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.

**• CONTENT OF STEREOISOMERS**

[NOTE—It is recommended to keep all solutions containing argatroban at about 4°.]

**Mobile phase:** Methanol and water (520:480)

**Standard solution:** 0.16 mg/mL of USP Argatroban RS in methanol

**Sample solution:** 0.16 mg/mL of Argatroban in methanol

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 259 nm

**Column:** 4.6-mm × 25-cm; 3-μm packing L1

**Column temperature:** 50°

**Flow rate:** 0.6 mL/min

**Injection volume:** 10 μL

**Run time:** NLT 1.4 times the retention time of (R)-argatroban

**System suitability**

**Sample:** Standard solution

**Suitability requirements**

**Resolution:** NLT 1.2 between (R)-argatroban and (S)-argatroban

**Relative standard deviation:** NMT 2.0% for (R)-argatroban and (S)-argatroban

**Analysis**

**Sample:** Sample solution

Calculate the percentage of (R)-argatroban and (S)-argatroban in the portion of Argatroban taken:

$$\text{Result} = [(r_U \text{ or } r_S)/(r_U + r_S)] \times 100$$

$r_U$  = peak response of (R)-argatroban from the *Sample solution*

$r_S$  = peak response of (S)-argatroban from the *Sample solution*

**Acceptance criteria:** See Table 5.

**Table 5**

Name	Relative Retention Time	Acceptance Criteria, (%)
(R)-Argatroban <sup>a</sup>	1.00	63–67
(S)-Argatroban <sup>b</sup>	1.06	33–37

<sup>a</sup> (2R,4R)-4-Methyl-1-[N<sup>2</sup>-{[(R)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-L-arginyl}]pipercolic acid.

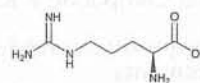
<sup>b</sup> (2R,4R)-4-Methyl-1-[N<sup>2</sup>-{[(S)-1,2,3,4-tetrahydro-3-methyl-8-quinolyl)sulfonyl]-L-arginyl}]pipercolic acid.

**SPECIFIC TESTS**

- **WATER DETERMINATION** (921), *Method Ia*: 3.0%–6.0%
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 2.0 Endotoxin Units/mg of argatroban
- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count is NMT 10<sup>2</sup> cfu/g, and the total combined molds and yeasts count is NMT 10<sup>2</sup> cfu/g.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Argatroban RS
  - USP Argatroban Related Compound A RS  
(2R,4R)-1-[N<sup>8</sup>-Nitro-N<sup>2</sup>-(3-methylquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.  
C<sub>23</sub>H<sub>31</sub>N<sub>7</sub>O<sub>7</sub>S 549.60
  - USP Argatroban Related Compound B RS  
Ethyl (2R,4R)-1-[N<sup>8</sup>-nitro-L-arginyl]-4-methylpiperidine-2-carboxylate hydrochloride.  
C<sub>15</sub>H<sub>28</sub>N<sub>6</sub>O<sub>5</sub> · HCl 408.88
  - USP Argatroban Related Compound C RS  
(2R,4R)-1-[N<sup>8</sup>-Amino-N<sup>2</sup>-(3-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonyl)-L-arginyl]-4-methylpiperidine-2-carboxylic acid.  
C<sub>23</sub>H<sub>37</sub>N<sub>7</sub>O<sub>5</sub>S 523.65
  - USP Endotoxin RS

**Arginine**

C<sub>6</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>

L-Arginine [74-79-3].

174.20

**DEFINITION**

Arginine contains NLT 98.5% and NMT 101.5% of C<sub>6</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>, as L-arginine, calculated on the dried basis.

**IDENTIFICATION**

- **INFRARED ABSORPTION** (197K)

**ASSAY**

- **PROCEDURE**

**Sample:** 80 mg of Arginine

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Potentiometric

**Blank:** 3 mL of formic acid and 50 mL of glacial acetic acid

**Analysis:** Dissolve the *Sample* in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate



with Titrant. Calculate the percentage of  $C_6H_{14}N_4O_2$  in the portion taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

- V = Sample titrant volume (mL)  
 B = Blank titrant volume (mL)  
 N = titrant normality (mEq/mL)  
 F = equivalency factor: 87.10 mg/mEq  
 W = weight of Sample (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

## IMPURITIES

### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.3%
- **CHLORIDE AND SULFATE, Chloride** (221): A 1.0-g portion shows no more chloride than corresponds to 0.70 mL of 0.020 N hydrochloric acid (0.05%).
- **CHLORIDE AND SULFATE, Sulfate** (221): A 1.0-g portion shows no more sulfate than corresponds to 0.30 mL of 0.020 N sulfuric acid (0.03%).
- **IRON** (241): NMT 30 ppm

### Delete the following:

- **HEAVY METALS, Method I** (231): NMT 15 ppm (Official 1: Jan-2018)

### Organic Impurities

#### PROCEDURE

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture  
**Standard solution:** 0.05 mg/mL of USP L-Arginine RS in 0.1 N hydrochloric acid. [NOTE—This solution has a concentration equivalent to 0.5% of that of the Sample solution.]  
**Sample solution:** 10 mg/mL of Arginine in 2 N hydrochloric acid  
**System suitability solution:** 0.4 mg/mL each of USP L-Arginine RS and USP L-Lysine Hydrochloride RS in 0.1 N hydrochloric acid  
**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)  
**Application volume:** 5  $\mu$ L  
**Developing solvent system:** Isopropyl alcohol and ammonium hydroxide (7:3)  
**Analysis**

**Samples:** Standard solution, Sample solution, and System suitability solution

Proceed as directed under *Chromatography* (621), *Thin-Layer Chromatography*. Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent*, and heat between 100° and 105° for about 15 min. Examine the plate under white light. The chromatogram obtained from the *System suitability solution* exhibits two clearly separated spots.

#### Acceptance criteria

**Individual impurities:** Any secondary spot from the *Sample solution* is not larger or more intense than the principal spot from the *Standard solution*, NMT 0.5%  
**Total impurities:** NMT 2.0%

### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation** (781S): +26.3° to +27.7°  
**Sample solution:** 80 mg/mL in 6 N hydrochloric acid
- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

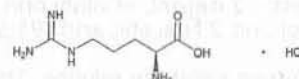
### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## USP REFERENCE STANDARDS (11)

- USP L-Arginine RS
- USP L-Lysine Hydrochloride RS

## Arginine Hydrochloride



$C_6H_{14}N_4O_2 \cdot HCl$  210.66  
 L-Arginine monohydrochloride;  
 L-(+)-Arginine monohydrochloride [1119-34-2].

### DEFINITION

Arginine Hydrochloride contains NLT 98.5% and NMT 101.5% of arginine hydrochloride ( $C_6H_{14}N_4O_2 \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

### ASSAY

#### PROCEDURE

**Sample:** 100 mg of Arginine Hydrochloride

#### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Potentiometric

**Blank:** 50 mL of glacial acetic acid and 3 mL of 98% formic acid. Add 6 mL of mercuric acetate TS.

**Analysis:** Dissolve the *Sample* in 3 mL of 98% formic acid and 50 mL of glacial acetic acid. Add 6 mL of mercuric acetate TS and titrate with the *Titrant*.

Calculate the percentage of arginine hydrochloride ( $C_6H_{14}N_4O_2 \cdot HCl$ ) in the *Sample* taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

- V = Sample titrant volume (mL)  
 B = Blank titrant volume (mL)  
 N = titrant normality (mEq/mL)  
 F = equivalency factor, 105.3 mg/mEq  
 W = weight of Sample (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **CHLORIDE AND SULFATE, Sulfate** (221): A 1.6-g portion shows no more sulfate than corresponds to 0.50 mL of 0.020 N sulfuric acid (0.03%).

### Delete the following:

- **HEAVY METALS, Method I** (231)

**Test preparation:** Proceed as directed in the chapter, except to dissolve 1.0 g in 20 mL of water, add 2 mL of 1 N acetic acid, and dilute with water to 25 mL.

**Acceptance criteria:** NMT 20 ppm (Official 1: Jan-2018)

#### CHROMATOGRAPHIC PURITY

**System suitability solution:** 0.4 mg/mL each of USP Arginine Hydrochloride RS and USP L-Lysine Hydrochloride RS in water

**Standard solution:** 0.05 mg/mL of USP Arginine Hydrochloride RS in water. [NOTE—This solution has a concentration equivalent to about 0.5% of that of the *Sample solution*.]

**Sample solution:** 10 mg/mL of Arginine Hydrochloride in water



**Chromatographic system**(See *Chromatography* (621), *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture**Application volume:** 5  $\mu$ L**Developing solvent system:** Isopropyl alcohol and ammonium hydroxide (70:30)**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)**Analysis****Samples:** *System suitability solution*, *Standard solution*, and *Sample solution*Proceed as directed in the chapter. Dry the plate between 100° and 105° until the ammonia disappears completely. Spray with *Spray reagent*, and heat between 100° and 105° for about 15 min. Examine the plate under white light. The *System suitability solution* exhibits two clearly separated spots.**Acceptance criteria:** Any secondary spot from the *Sample solution* is not larger or more intense than the principal spot from the *Standard solution*.**Individual impurities:** NMT 0.5%**Total impurities:** NMT 2.0%**SPECIFIC TESTS**

- OPTICAL ROTATION**, *Specific Rotation* (781S): +21.4° to +23.6° ( $t = 20^\circ$ )  
*Sample solution:* 80 mg/mL in 6 N hydrochloric acid
- LOSS ON DRYING** (731): Dry a sample at 105° for 2 h: it loses NMT 0.2% of its weight.

**Change to read:**

- CHLORIDE CONTENT**

**Sample:** 350 mg of Arginine Hydrochloride**Titrimetric system**(See *Titrimetry* (541).)**Mode:** Direct titration**Titrant:** 0.1 N silver nitrate VS**Endpoint detection:** Colorimetric

(ERR 1-Apr-2016)

**Analysis:** Transfer the *Sample* to a porcelain casserole, and add 140 mL of water and 1 mL of dichlorofluorescein TS. Mix and titrate with the *Titrant* until the silver chloride flocculates and the mixture acquires a faint pink color.Calculate the percentage of chloride (Cl) in the *Sample* taken:

$$\text{Result} = (V \times N \times F \times 100) / W \quad (\text{ERR 1-Apr-2016})$$

 $V$  = *Sample* titrant volume (mL)

(ERR 1-Apr-2016)

 $N$  = titrant normality (mEq/mL) $F$  = equivalency factor, 35.45 mg/mEq $W$  = weight of *Sample* (mg)**Acceptance criteria:** 16.5%–17.1%**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers.
- USP REFERENCE STANDARDS** (11)  
USP Arginine Hydrochloride RS  
USP L-Lysine Hydrochloride RS

jection. It contains not less than 9.5 percent and not more than 10.5 percent of  $\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2 \cdot \text{HCl}$ . It contains no antimicrobial agents.

**NOTE**—The chloride ion content of Arginine Hydrochloride Injection is approximately 475 mEq per L.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type II glass.

**USP Reference standards** (11)—

USP Arginine Hydrochloride RS

USP Endotoxin RS

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**Identification**—

**A:** Transfer 1 mL of the Injection to a 200-mL volumetric flask, and dilute with water to volume. To 1 mL of this dilution add 2 mL of a solution of 0.02% 8-hydroxyquinoline in 3 N sodium hydroxide, and add 1 mL of 0.1% *N*-bromosuccinimide solution: an orange color is produced.

**B:** It meets the requirements of the tests for *Chloride* (191).

**Bacterial Endotoxins Test** (85)—It contains not more than 0.01 USP Endotoxin Unit per mg of arginine hydrochloride.

**pH** (791): between 5.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections* and *Implanted Drug Products* (1).

**Assay**—

**Color reagent**—Dissolve 28.0 g of potassium hydroxide and 2.0 g of potassium sodium tartrate in 100 mL of water. Cool, and add, in the order named, 100 mg of 2,4-dichloro-1-naphthol, 180 mL of alcohol, and 20.0 mL of 0.475% sodium hypochlorite solution. Mix by swirling, and allow to stand at room temperature for 1 hour before using. This *Color reagent* may be stored in a glass-stoppered bottle, in a refrigerator, for 2 months.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Arginine Hydrochloride RS in water, and dilute quantitatively and stepwise with water to obtain a solution having a known concentration of about 40  $\mu$ g per mL.

**Assay preparation**—Pipet into a 100-mL volumetric flask a volume of Injection, equivalent to 200 mg of arginine hydrochloride, add water to volume, and mix. Pipet 5 mL of this solution into a 250-mL volumetric flask, add water to volume, and mix.

**Procedure**—Transfer 2.0-mL portions of the *Assay preparation* and the *Standard preparation*, respectively, to separate flasks, and treat each as follows. Add 2.0 mL of potassium iodide solution (3 in 1000), mix, and allow to stand for 15 minutes. Add 6.0 mL of *Color reagent*, mix, and allow to stand for 15 minutes. Add 2.0 mL of sodium hypochlorite solution (19 in 10,000), mix, and allow to stand for 15 minutes. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 520 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2 \cdot \text{HCl}$  in each mL of the Injection taken by the formula:

$$5(C/V)(A_U/A_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP Arginine Hydrochloride RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the

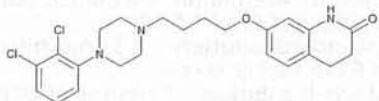
**Arginine Hydrochloride Injection**

» Arginine Hydrochloride Injection is a sterile solution of Arginine Hydrochloride in Water for In-



absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Aripiprazole



$C_{23}H_{27}Cl_2N_3O_2$  448.39  
2(1*H*)-Quinolinone, 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydro-;  
7-[4-[4-(2,3-Dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydrocarbostyryl [129722-12-9].

### DEFINITION

Aripiprazole contains NLT 98.0% and NMT 102.0% of aripiprazole ( $C_{23}H_{27}Cl_2N_3O_2$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

Protect the solutions from light.

**Diluent:** Acetonitrile, methanol, water, and acetic acid (30:10:60:1)

**Solution A:** Acetonitrile and 0.05% trifluoroacetic acid (10:90)

**Solution B:** Acetonitrile and 0.05% trifluoroacetic acid (90:10)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	80	20
2	80	20
10	65	35
20	10	90
25	10	90
26	80	20
35	80	20

[NOTE—The gradient was established on an HPLC system with a dwell volume of approximately 650  $\mu$ L.]

**System suitability solution:** 1  $\mu$ g/mL each of USP Aripiprazole RS and USP Aripiprazole Related Compound F RS in *Diluent*

**Standard solution:** 0.1 mg/mL of USP Aripiprazole RS in *Diluent*

**Sample solution:** 0.1 mg/mL of Aripiprazole in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  10-cm; 3- $\mu$ m packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for aripiprazole and aripiprazole related compound F are 1.0 and 1.1, respectively.]

### Suitability requirements

**Resolution:** NLT 2.0 between aripiprazole and aripiprazole related compound F, *System suitability solution*

**Tailing factor:** NMT 1.5 for aripiprazole, *System suitability solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aripiprazole ( $C_{23}H_{27}Cl_2N_3O_2$ ) in the portion of Aripiprazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aripiprazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

### Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 10 ppm (Official 1-

Jan-2018)

- **ORGANIC IMPURITIES**

Protect the solutions from light.

**Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Aripiprazole taken:

$$\text{Result} = (r_i/r_U) \times (1/F) \times 100$$

$r_i$  = peak response of each impurity from the *Sample solution*

$r_U$  = peak response of Aripiprazole from the *Sample solution*

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Aripiprazole related compound G <sup>a</sup>	0.9	0.72	0.10
Aripiprazole	1.0	—	—
Aripiprazole related compound F <sup>b,c</sup>	1.1	1.0	0.10
Aripiprazole 4,4'-dimer <sup>d</sup>	1.3	1.0	0.10

<sup>a</sup> 7-[4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy]quinolin-2(1*H*)-one.

<sup>b</sup> 4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yl)oxy]butyl]piperazine 1-oxide.

<sup>c</sup> If possible from the manufacturing process.

<sup>d</sup> 1,1'-(Ethane-1,1-diyl)bis(2,3-dichloro-4-[4-[3,4-dihydroquinolin-2(1*H*)-one-7-yloxybutyl]piperazin-1-yl]benzene).



Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.50

<sup>a</sup> 7-[4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butoxy]quinolin-2(1*H*)-one.

<sup>b</sup> 4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazine 1-oxide.

<sup>c</sup> If possible from the manufacturing process.

<sup>d</sup> 1,1'-(Ethane-1,1-diyl)bis(2,3-dichloro-4-(4-[3,4-dihydroquinolin-2(1*H*)-one-7-yloxybutyl]piperazin-1-yl)benzene).

### SPECIFIC TESTS

#### • LOSS ON DRYING (731)

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 0.5%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

#### • USP REFERENCE STANDARDS (11)

USP Aripiprazole RS

USP Aripiprazole Related Compound F RS

4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazine 1-oxide.

C<sub>23</sub>H<sub>27</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>3</sub> 464.38

## Aripiprazole Tablets

### DEFINITION

Aripiprazole Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of aripiprazole (C<sub>23</sub>H<sub>27</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Standard:** Add 30 mL of ethyl acetate to 30 mg of USP Aripiprazole RS. Shake for 10 min, centrifuge for NLT 5 min, and pass the supernatant through a suitable membrane filter. To the filtrate add 15 mL of water, shake for 5 min, and centrifuge for NLT 10 min. Transfer 20 mL of the upper layer to a container and add anhydrous magnesium sulfate, as needed. Shake well, pass through a suitable membrane filter, and evaporate the ethyl acetate on a water bath under reduced pressure. Use the residue. [NOTE—A centrifuge speed of 2000 rpm may be suitable.]

**Sample:** Grind a suitable number of Tablets and transfer a suitable portion of the ground Tablets, equivalent to 30 mg of aripiprazole, to an appropriate container. Add 30 mL of ethyl acetate, shake for 10 min, centrifuge for NLT 5 min, and pass the supernatant through a suitable membrane filter. To the filtrate add 15 mL of water, shake for 5 min, and centrifuge for NLT 10 min. Transfer 20 mL of the upper layer to a container and add a suitable amount of anhydrous magnesium sulfate. Shake well, pass through a suitable membrane filter, and evaporate the ethyl acetate on a water bath under reduced pressure. Use the residue. [NOTE—A centrifuge speed of 2000 rpm may be suitable.]

#### Analysis

Samples: *Standard* and *Sample*

Acceptance criteria: Meet the requirements

- **B.** The retention time of the aripiprazole peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### Change to read:

#### • PROCEDURE

**Solution A:** 2.8 g/L of anhydrous sodium sulfate (RB 1, Jun-2016) in water

**Mobile phase:** Acetonitrile, methanol, *Solution A*, and glacial acetic acid (33:11:56:1)

**Internal standard solution:** 0.33 mg/mL of USP Propylparaben RS in *Mobile phase*

**Standard stock solution:** 1 mg/mL of USP Aripiprazole RS in *Mobile phase*

**Standard solution:** 0.2 mg/mL of USP Aripiprazole RS prepared as follows. Transfer 10.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample solution:** Nominally 0.2 mg/mL of aripiprazole from Tablets prepared as follows. Powder NLT 20 Tablets and transfer a suitable portion of the powder to an appropriate volumetric flask. Add 40% of the final flask volume of *Mobile phase* and 20% of the final flask volume of *Internal standard solution*. Shake for 10 min, and dilute with *Mobile phase* to volume. Centrifuge, if necessary, and pass the supernatant through a suitable filter of NMT 0.5-μm pore size, discard the first 1 mL of filtrate, and use the subsequent filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**Run time:** NLT 2 times the retention time of aripiprazole

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for aripiprazole and propylparaben are about 1.0 and 1.5, respectively.]

#### Suitability requirements

**Resolution:** NLT 8 between aripiprazole and propylparaben

**Tailing factor:** NMT 1.7 for aripiprazole and for propylparaben

**Relative standard deviation:** NMT 2.0% for the peak response ratio of aripiprazole to propylparaben

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aripiprazole (C<sub>23</sub>H<sub>27</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of aripiprazole to propylparaben from the *Sample solution*

$R_S$  = peak response ratio of aripiprazole to propylparaben from the *Standard solution*

$C_S$  = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aripiprazole in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

### PERFORMANCE TESTS

#### Change to read:

#### • DISSOLUTION (711)

**Medium:** pH 1.2 hydrochloric acid buffer (Transfer 250 mL of 14.9 g/L of potassium chloride in water to a 1-L volumetric flask, add 425 mL of 0.2 N hydrochloric acid.)



ric acid, (RB 1-Jun-2016) and dilute with water to volume. Degas the resulting solution or pass the resulting solution through a filter under vacuum., degassed; 900 mL

Apparatus 2: 60 rpm

Time: 30 min

**Procedure:** Determine the percentage of the labeled amount of aripiprazole ( $C_{23}H_{27}Cl_2N_3O_2$ ) dissolved by using either the *Spectrometric procedure* or the *Chromatographic procedure* described below.

#### Spectrometric procedure

**Standard stock solution:** 1 mg/mL of USP

Aripiprazole RS in alcohol

**Standard solution:** (L/900) mg/mL of USP

Aripiprazole RS from *Standard stock solution* in *Medium*, where L is the label claim, in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter, discarding the first 5 mL of filtrate.

#### Instrumental conditions

**Mode:** UV

**Analytical wavelengths:** 249 and 325 nm

**Cell length:** 1 cm

**Blank:** *Medium*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aripiprazole ( $C_{23}H_{27}Cl_2N_3O_2$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

$A_U$  = absorbance at 249 nm minus the absorbance at 325 nm of the *Sample solution*

$A_S$  = absorbance at 249 nm minus the absorbance at 325 nm of the *Standard solution*

$C_S$  = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

#### Chromatographic procedure

**Solution A:** 2.8 g/L of anhydrous sodium sulfate, (RB 1-Jun-2016)

**Solution B:** 13.9 g/L of glacial acetic acid and 23.9 g/L of sodium acetate, (RB 1-Jun-2016) in water

**Mobile phase:** Acetonitrile, methanol, *Solution A*, and glacial acetic acid (40:10:50:1)

**Diluent:** *Solution B* and methanol (50:50)

**Internal standard solution:** 0.67 µg/mL of USP Propylparaben RS in *Diluent*, (RB 1-Jun-2016)

**Standard stock solution A:** 1 mg/mL of USP Aripiprazole RS in *Mobile phase*

**Standard stock solution B:** 0.002 mg/mL of USP Aripiprazole RS from *Standard stock solution A* in *Medium* passed through a suitable filter of NMT 0.5-µm pore size, discarding the first 6 mL of filtrate

**Standard solution:** 0.001 mg/mL of USP Aripiprazole RS from *Standard stock solution B* prepared by combining 5 mL of *Standard stock solution B* and 5 mL of *Internal standard solution*

**Sample stock solution:** Pass a portion of the solution under test through a suitable filter of NMT 0.5-µm pore size, discarding NLT the first 6 mL of filtrate.

**Sample solution:** Combine 2 mL of *Sample stock solution* with 2 mL of *Internal standard solution*.

**Chromatographic system:** Proceed as directed in the Assay except as follows.

**Injection volume:** 100 µL

**Run time:** NLT 2 times the retention time of aripiprazole

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for aripiprazole and propylparaben are about 1.0 and 1.8, respectively.]

#### Suitability requirements

**Resolution:** NLT 10 between aripiprazole and propylparaben

**Relative standard deviation:** NMT 1.5% for the peak response ratio of aripiprazole to propylparaben

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aripiprazole ( $C_{23}H_{27}Cl_2N_3O_2$ ) dissolved:

$$\text{Result} = (R_U/R_S) \times C_S \times V \times (1/L) \times 100$$

$R_U$  = peak response ratio of aripiprazole to propylparaben from the *Sample solution*

$R_S$  = peak response ratio of aripiprazole to propylparaben from the *Standard solution*

$C_S$  = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of aripiprazole ( $C_{23}H_{27}Cl_2N_3O_2$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### Change to read:

#### • ORGANIC IMPURITIES

Protect solutions from light.

**Buffer:** 9.6 g/L of dibasic ammonium citrate, 1.6 g/L of citric acid, and 2.9 g/L of sodium dodecyl sulfate, (RB 1-Jun-2016) in water. Adjust with 11 g/L of dibasic ammonium citrate in water, (RB 1-Jun-2016) or 9.6 g/L of anhydrous citric acid in water, (RB 1-Jun-2016) to a pH of 4.7, if needed.

**Mobile phase:** Acetonitrile and *Buffer* (45:55)

**Diluent:** Acetonitrile, water, and glacial acetic acid (40:60:1)

**System suitability solution:** 0.5 mg/mL of USP Aripiprazole RS, and 0.0005 mg/mL each of USP Aripiprazole Related Compound F RS and USP Aripiprazole Related Compound G RS in *Diluent*

**Sample solution:** Nominally 0.5 mg/mL of aripiprazole from Tablets prepared as follows. Powder NLT 20 Tablets, transfer a suitable portion of the powder equivalent to NLT 4 mg of aripiprazole to an appropriate container, and add a suitable volume of *Diluent*. Shake for 10 min and centrifuge, if necessary. Pass the supernatant through a suitable filter of NMT 0.5-µm pore size, discard the first 1 mL of filtrate, and use the subsequent filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**Run time:** NLT 2 times the retention time of aripiprazole

#### System suitability

**Sample:** *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 3 between aripiprazole related compound G and aripiprazole

**Signal-to-noise ratio:** NLT 10 for aripiprazole related compound F and aripiprazole related compound G

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$



- $r_U$  = peak response of each degradation product from the *Sample solution*  
 $r_T$  = sum of all the peak responses from the *Sample solution*  
**Acceptance criteria:** See Table 1. • Disregard peaks that are less than 0.1% of the aripiprazole peak. • (RB 1-Jun-2016)

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aripiprazole related compound F	0.54	0.3 • (RB 1-Jun-2016)
Aripiprazole related compound G	0.81	0.3 • (RB 1-Jun-2016)
Aripiprazole	1.0	—
Any individual unspecified degradation product	—	0.2 • (RB 1-Jun-2016)
Total degradation products	—	1.0

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
  - USP Aripiprazole RS
  - USP Aripiprazole Related Compound F RS
  - 4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazine 1-oxide.  
 $C_{23}H_{27}Cl_2N_3O_3$  464.38
  - USP Aripiprazole Related Compound G RS
  - 7-[4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy]quinolin-2(1H)-one.  
 $C_{23}H_{25}Cl_2N_3O_2$  446.37
  - USP Propylparaben RS

## Aripiprazole Orally Disintegrating Tablets

**DEFINITION**

Aripiprazole Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of aripiprazole ( $C_{23}H_{27}Cl_2N_3O_2$ ).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197A)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Solution A:** 2.84 g/L of sodium sulfate in water  
**Buffer:** 3.48 g/L of dibasic potassium phosphate adjusted with phosphoric acid to a pH of 8.2  
**Mobile phase:** Acetonitrile and *Buffer* (50:50)  
**Diluent A:** Acetonitrile, methanol, *Solution A*, and glacial acetic acid (33:11:56:1)  
**Diluent B:** Acetonitrile and 0.1 M hydrochloric acid (20:80)  
**System suitability solution:** 0.01 mg/mL each of USP Aripiprazole RS and USP Aripiprazole Related Compound G RS in *Diluent A*. Sonication and shaking may be used to aid in dissolution.  
**Standard solution:** 0.25 mg/mL of USP Aripiprazole RS in *Diluent B*. Sonication may be used to aid in dissolution.  
**Sample solution:** Nominally 0.2–0.3 mg/mL of aripiprazole from NLT 5 Orally Disintegrating Tablets

prepared as follows. Transfer NLT 5 Orally Disintegrating Tablets to a suitable volumetric flask and dilute with *Diluent B* to NMT 75% of the final flask volume. Sonicate for 5 min and shake for 15 min. Dilute with *Diluent B* to volume. Pass the resulting solution through a suitable filter and use the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 252 nm

**Column:** 4.6-mm × 10-cm; 3.5-μm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**Run time:** NLT 1.4 times the retention time of aripiprazole

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times of aripiprazole related compound G and aripiprazole are 0.74 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.0 between aripiprazole related compound G and aripiprazole, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aripiprazole ( $C_{23}H_{27}Cl_2N_3O_2$ ) in the portion of Orally Disintegrating Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aripiprazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

• **DISINTEGRATION (701):** NMT 60 s

• **DISSOLUTION (711)**

**Medium:** pH 4.0 sodium acetate trihydrate buffer (3.0 g/L of sodium acetate prepared as follows. Transfer a suitable quantity of sodium acetate to a suitable container containing 90% of the final container volume of water. Adjust with glacial acetic acid to a pH of 4.0. Add water to the final volume.), degassed; 1000 mL

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Mobile phase:** Acetonitrile and 0.025 M hydrochloric acid (40:60)

**Standard solution:** (L/1000) mg/mL of USP Aripiprazole RS in *Mobile phase* where L is the label claim in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter, discarding the first few mL.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 225 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 μL

**Run time:** NLT 1.5 times the retention time of aripiprazole



**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of aripiprazole ( $C_{23}H_{27}Cl_2N_3O_2$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times (1/L) \times 100$$

 $r_u$  = peak response from the *Sample solution* $r_s$  = peak response from the *Standard solution* $C_s$  = concentration of USP Aripiprazole RS in the *Standard solution* (mg/mL) $V$  = volume of *Medium*, 1000 mL $L$  = label claim (mg/Tablet)**Tolerances:** NLT 80% (Q) of the labeled amount of aripiprazole ( $C_{23}H_{27}Cl_2N_3O_2$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES**

- **ORGANIC IMPURITIES**

**Solution A:** Water and trifluoroacetic acid (100:0.05)**Solution B:** Acetonitrile and trifluoroacetic acid (100:0.05)**Solution C:** 2.84 g/L of sodium sulfate in water**Mobile phase:** See *Table 1*.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	90	10
20	70	30
40	42	58
50	10	90
55	10	90
56	90	10
60	90	10

[NOTE—The gradient was established on an HPLC system with a dwell volume of approximately 1.0 mL.]

**Diluent:** Acetonitrile, methanol, *Solution C*, and glacial acetic acid (33:11:56:1)**System suitability solution:** 250 µg/mL of USP Aripiprazole RS, and 0.5 µg/mL each of USP Aripiprazole Related Compound F RS and USP Aripiprazole Related Compound G RS in *Diluent*. Sonication may be used to aid in dissolution.**Sample solution:** Nominally 0.2–0.3 mg/mL of aripiprazole from NLT 5 Orally Disintegrating Tablets prepared as follows. Transfer NLT 5 Orally Disintegrating Tablets to a suitable volumetric flask. Add about 70% of the total volume of *Diluent*. Sonicate for 10 min and shake for 10 min. Dilute with *Diluent* to volume. Pass the resulting solution through a suitable filter and use the filtrate.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 15-cm; 3-µm packing L1**Flow rate:** 1 mL/min**Injection volume:** 20 µL**System suitability****Sample:** *System suitability solution*[NOTE—See *Table 2* for the relative retention times.]**Suitability requirements****Resolution:** NLT 4.0 between aripiprazole related compound G and aripiprazole; NLT 1.5 between aripiprazole and aripiprazole related compound F**Analysis****Sample:** *Sample solution*Calculate the total peak response for individual impurities and aripiprazole from the *Sample solution*:

$$\text{Result} = \Sigma[r_i \times (1/F)] + r_u$$

 $r_i$  = peak response of each degradation product from the *Sample solution* $F$  = relative response factor (see *Table 2*) $r_u$  = peak response of aripiprazole from the *Sample solution*

Calculate the percentage of each degradation product in the portion of Orally Disintegrating Tablets taken:

$$\text{Result} = (r_i/r_T) \times (1/F) \times 100$$

 $r_i$  = peak response of each degradation product from the *Sample solution* $r_T$  = total peak response for individual impurities and aripiprazole from the *Sample solution* $F$  = relative response factor (see *Table 2*)**Acceptance criteria:** See *Table 2*. Disregard peaks less than 0.05%.**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Aripiprazole related compound G	0.96	0.77	0.3
Aripiprazole	1.0	—	—
Aripiprazole related compound F	1.03	1.0	0.3
Any individual unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	1.0

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

- **USP REFERENCE STANDARDS (11)**

USP Aripiprazole RS

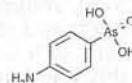
USP Aripiprazole Related Compound F RS

4-(2,3-Dichlorophenyl)-1-[4-(2-oxo-1,2,3,4-tetrahydroquinolin-7-yloxy)butyl]piperazine 1-oxide.

 $C_{23}H_{27}Cl_2N_3O_3$  464.38

USP Aripiprazole Related Compound G RS

7-[4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butoxy]quinolin-2(1H)-one.

 $C_{23}H_{25}Cl_2N_3O_2$  446.37**Arsanilic Acid** $C_6H_8AsNO_3$  217.05*p*-Aminobenzenearsonic acid [98-50-0].» Arsanilic Acid contains not less than 98.0 percent and not more than 102.0 percent of  $C_6H_8AsNO_3$ , calculated on the dried basis.



**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Arsanilic Acid RS

**Identification**, *Infrared Absorption* (197K).

**Loss on drying** (731)—Dry it in vacuum at 80° for 4 hours; it loses not more than 0.5% of its weight.

**Limit of o-arsanilic acid**—

**Mobile phase**—Dissolve 4.04 g of monobasic potassium phosphate in 985 mL of water, add 2 mL of phosphoric acid, and mix. Add 10 mL of methanol, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Transfer about 67 mg of o-arsanilic acid, accurately weighed, to a 100-mL volumetric flask, add about 65 mg of warm (about 70° to 80°) water, and shake or sonicate to dissolve. Allow to cool, dilute with water to volume, and mix. Dilute a portion of this solution quantitatively and stepwise with water to obtain a solution having a known concentration of about 0.0012 mg of o-arsanilic acid per mL.

**Test solution**—Transfer about 50 mg of Arsanilic Acid, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of warm water, and shake or sonicate to dissolve. Allow to cool, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 242-nm detector and a 4.6-mm × 15-cm column that contains 5-μm base-deactivated packing L1 and is maintained at a constant temperature of about 30°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factor, *k'*, for the o-arsanilic acid peak is between 2.8 and 3.8; and the relative standard deviation for replicate injections is not more than 2.5%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the responses for the o-arsanilic acid peaks. Calculate the percentage of o-arsanilic acid in the portion of Arsanilic Acid taken by the formula:

$$5000(C/W)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of o-arsanilic acid in the *Standard solution*; *W* is the weight, in mg, of Arsanilic Acid taken to prepare the *Test solution*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the responses of the o-arsanilic acid peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.12% is found.

**Limit of aniline**—

**Mobile phase**—Dissolve 7.76 g of monobasic potassium phosphate in 950 mL of water, add 50 mL of methanol, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Transfer about 176 mg of aniline, accurately weighed, to a 25-mL volumetric flask, add about 1 mL of methanol, swirl, then add about 15 mL of water, and shake to dissolve. Dilute with water to volume, and mix. Dilute a portion of this solution quantitatively and stepwise with water to obtain a solution having a known concentration of about 0.00045 mg of aniline per mL.

**Test solution**—Transfer about 50 mg of Arsanilic Acid, accurately weighed, to a 50-mL volumetric flask, add about 30 mL of warm (about 70° to 80°) water, and shake or sonicate to dissolve. Allow to cool, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 235-nm detector

and a 4.6-mm × 15-cm column that contains 5-μm base-deactivated packing L1 and is maintained at a constant temperature of about 30°. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the capacity factor, *k'*, for the aniline peak is between 2.3 and 3.3; and the relative standard deviation for replicate injections is not more than 3.0%.

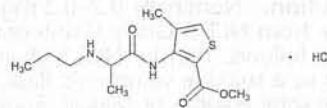
**Procedure**—Separately inject equal volumes (about 50 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the responses for the aniline peaks. Calculate the percentage of aniline in the portion of Arsanilic Acid taken by the formula:

$$5000(C/W)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of aniline in the *Standard solution*; *W* is the weight, in mg, of Arsanilic Acid taken to prepare the *Test solution*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the responses of the aniline peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.045% is found.

**Assay**—Transfer about 125 mg of Arsanilic Acid, accurately weighed, to a 50-mL conical flask, and add 10.0 mL of a mixture of sulfuric acid, nitric acid, and perchloric acid (1000:50:50) and several glass beads. Digest on a hot plate for about 1 hour, increasing the temperature of the hot plate in steps until a ring of sulfuric acid rises into the neck of the flask. Allow to cool, to the colorless solution add about 400 mg of hydrazine sulfate, and heat the flask vigorously on a hot plate until a ring of sulfuric acid rises into the neck of the flask. Allow to cool, and wash down the rim, neck, and insides of the flask with about 1 mL of water. Heat the flask again until a ring of sulfuric acid rises into the neck of the flask. Allow to cool, and transfer the colorless solution, with the aid of about 80 mL of water, to a 125-mL conical flask. Add 10 mL of hydrochloric acid and several drops of 0.002 M potassium iodide, cool to between 0° and 5°, and titrate with 0.1 N potassium permanganate VS to a pale pink endpoint, maintaining the temperature between 0° and 5° during the titration. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N potassium permanganate is equivalent to 10.852 mg of C<sub>6</sub>H<sub>8</sub>AsNO<sub>3</sub>.

## Articaine Hydrochloride



C<sub>13</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>S · HCl 320.84

2-Thiophenecarboxylic acid, 4-methyl-3-[[1-oxo-2-(propylamino)propyl]amino]-, methyl ester, monohydrochloride;

Methyl 4-methyl-3-[2-(propylamino)propionamido]-2-thiophenecarboxylate, monohydrochloride [23964-57-0].

### DEFINITION

Articaine Hydrochloride contains NLT 98.5% and NMT 101.0% of C<sub>13</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub>S · HCl, calculated on the dried basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197)

**Standard solution:** 12 mg/mL of USP Articaine RS in methylene chloride. Transfer 20 μL of this solution onto a 300-mg disk.



**Sample solution:** Dissolve 100 mg of Articaine Hydrochloride in 5 mL of water. Add 3 mL of a saturated solution of sodium bicarbonate, and shake twice with 2 mL of methylene chloride. Combine the methylene chloride layers, dilute with methylene chloride to 5.0 mL, and dry over anhydrous sodium sulphate. Transfer 20  $\mu$ L of this solution onto a 300-mg disk.

• **B. IDENTIFICATION TESTS—GENERAL, Chloride (191)**

**ASSAY**

• **PROCEDURE**

**Sample solution:** 250 mg of Articaine Hydrochloride to a 250-mL conical flask. Add 5.0 mL of 0.01 M hydrochloric acid and 50 mL of alcohol. Stir to dissolve.

**Analysis:** Titrate with 0.1 M sodium hydroxide VS, determining the endpoint potentiometrically, using a glass electrode. Calculate the volume of sodium hydroxide consumed by reading the volume added between the two points of inflection. Each mL of 0.1 M sodium hydroxide is equivalent to 32.08 mg of  $C_{13}H_{20}N_2O_3S \cdot HCl$ .  
Acceptance criteria: 98.5%–101.0% on the dried basis.

**IMPURITIES**

**Inorganic Impurities**

Delete the following:

• **HEAVY METALS, Method I (231)**

**Sample solution:** 200 mg/mL of Articaine Hydrochloride

Acceptance criteria: NMT 5 ppm (Official 1-Jan-2018)

• **RESIDUE ON IGNITION (281)**

**Sample:** 1 g

Acceptance criteria: NMT 0.1%

**Organic Impurities**

• **PROCEDURE**

**Buffer solution:** 2.02 g of sodium 1-heptanesulfonate and 4.08 g of potassium dihydrogen phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 2.0.

**Mobile phase:** Acetonitrile and Buffer solution (1:3)

**Standard solution:** 2  $\mu$ g/mL of USP Articaine Related Compound A, and 1  $\mu$ g/mL each of USP Articaine Related Compound E RS and USP Articaine Hydrochloride RS in Mobile phase. [NOTE—This solution is also used to determine the reporting threshold limit.]

**Sample solution:** 1.0 mg/mL of Articaine Hydrochloride in Mobile phase

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 276 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Temperature:** 45°

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

**System suitability**

**Sample:** Standard solution

**Suitability requirements**

**Resolution:** NLT 1.2 between articaine related compound A and articaine related compound E

**Analysis**

**Samples:** Standard solution and Sample solution.

[NOTE—Run time is 5 times the retention time of articaine.]

Calculate the percentage of any articaine related compound A in the portion of Articaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = response of articaine related compound A from the Sample solution

$r_S$  = response of articaine related compound A from the Standard solution

$C_S$  = concentration of USP Articaine Related Compound A RS in the Standard solution (mg/mL)

$C_U$  = concentration of Articaine Hydrochloride in the Sample solution (mg/mL)

Calculate the percentage of each individual impurity in the portion of Articaine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = response of each individual impurity from the Sample solution

$r_S$  = response of articaine hydrochloride from the Standard solution

$C_S$  = concentration of USP Articaine Hydrochloride RS in the Standard solution (mg/mL)

$C_U$  = concentration of Articaine Hydrochloride in the Sample solution (mg/mL)

[NOTE—Disregard any peak below 0.05%.]

**Acceptance criteria**

**Individual impurities:** See Impurity Table 1.

**Total impurities:** NMT 0.5%. [NOTE—Excluding articaine related compound A.]

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Articaine acid <sup>a</sup>	0.6	0.1
Ethylarticaine <sup>b</sup>	0.7	0.1
Articaine related compound A <sup>c</sup>	0.8	0.2
Articaine related compound E <sup>d</sup>	0.86	0.1
Articaine acid-propionamide <sup>e</sup>	0.9	0.1
Articaine	1.0	—
Butylarticaine <sup>f</sup>	1.7	0.1
Dipropylarticaine <sup>g</sup>	2.1	0.1
3-Aminoarticaine <sup>h</sup>	2.6	0.1
Articaine isopropyl ester <sup>i</sup>	3.6	0.1
Bromo compound <sup>j</sup>	4.0	0.1
Any other individual impurity	—	0.10

<sup>a</sup> 4-Methyl-3-[[[(2RS)-2-(propylamino)propanoyl]amino]thiophene-2-carboxylic acid.

<sup>b</sup> Methyl 3-[[[(2RS)-2-(ethylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.

<sup>c</sup> Methyl 4-methyl-3-[2-(propylamino)acetamido]thiophene-2-carboxylate.

<sup>d</sup> Methyl 3-[2-(isopropylamino)propanamido]-4-methylthiophene-2-carboxylate.

<sup>e</sup> 4-Methyl-N-propyl-3-[[[(2RS)-2-(propylamino)propanoyl]amino]thiophene-2-carboxamide.

<sup>f</sup> Methyl 3-[[[(2RS)-2-(butylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.

<sup>g</sup> Methyl 3-[[[(2RS)-2-(dipropylamino)propanoyl]amino]-4-methylthiophene-2-carboxylate.

<sup>h</sup> Methyl 3-amino-4-methylthiophene-2-carboxylate.

<sup>i</sup> 1-Methylethyl 4-methyl-3-[[[(2RS)-2-(propylamino)propanoyl]amino]thiophene-2-carboxylate.

<sup>j</sup> Methyl 3-[[[(2RS)-2-bromopropanoyl]amino]-4-methylthiophene-2-carboxylate.

**SPECIFIC TESTS**

• **LOSS ON DRYING (731):** Dry at 105° for 5 h: it loses NMT 0.5% of its weight.

• **PH (791)**

**Sample solution:** 10 mg/mL

**Acceptance criteria:** 4.2–5.2

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in light-resistant containers.



• **USP REFERENCE STANDARDS** (11)

- USP Articaine RS  
 USP Articaine Hydrochloride RS  
 USP Articaine Related Compound A RS  
 Methyl 4-methyl-3-[2-(propylamino) acetamido]thiophene-2-carboxylate.  
 $C_{12}H_{18}N_2O_3S$  270.35  
 USP Articaine Related Compound E RS  
 Methyl 3-[2-(isopropylamino) propanamido]-4-methylthiophene-2-carboxylate.  
 $C_{13}H_{20}N_2O_3S$  284.37

## Articaine Hydrochloride and Epinephrine Injection

### DEFINITION

Articaine Hydrochloride and Epinephrine Injection is a sterile solution of Articaine Hydrochloride and Epinephrine, in Water for Injection, and contains NLT 95.0% and NMT 105.0% of the labeled amount of articaine hydrochloride ( $C_{13}H_{20}N_2O_3S \cdot HCl$ ) and NLT 90.0% and NMT 115.0% of the labeled amount of epinephrine ( $C_9H_{13}NO_3$ ).

### IDENTIFICATION

- **A.** The retention times of the articaine and epinephrine peaks from the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assays for Articaine Hydrochloride and Epinephrine*, respectively.

### ASSAY

• **ARTICAINE HYDROCHLORIDE**

**Buffer:** Glacial acetic acid and water (50:930). Adjust with 2 N sodium hydroxide to a pH of 3.4.  
**Mobile phase:** Acetonitrile and *Buffer* (22:78)  
**Standard stock solution:** 40 mg/mL of USP Articaine Hydrochloride RS in water  
**Standard solution:** 0.8 mg/mL of USP Articaine Hydrochloride RS in *Mobile phase* from *Standard stock solution*  
**Sample solution:** Equivalent to 0.8 mg/mL of articaine hydrochloride in *Mobile phase* from a portion of Injection  
**Chromatographic system**  
 (See *Chromatography* (621), *System Suitability*).  
**Mode:** LC  
**Detector:** UV 270 nm  
**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1  
**Flow rate:** 1 mL/min  
**Injection size:** 10  $\mu$ L  
**Run time:** 2.5 times the retention time of articaine

#### System suitability

**Sample:** *Standard solution*  
**Suitability requirements**  
**Tailing factor:** NMT 2.2  
**Relative standard deviation:** NMT 1.0%, from six injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of articaine hydrochloride ( $C_{13}H_{20}N_2O_3S \cdot HCl$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Articaine Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of articaine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

• **EPINEPHRINE**

**Mobile phase:** Mix 50 mL of glacial acetic acid and 930 mL of water. Adjust with 2 N sodium hydroxide to a pH of 3.4. In this solution, dissolve 1.2 g of sodium 1-heptanesulfonate, and add 1.0 mL of 0.1 M edetate disodium and 0.298 g of potassium chloride. Add 150 mL of methanol.

**Diluent:** 0.5 mg/mL potassium metabisulfite in water  
**System suitability solution:** 22  $\mu$ g/mL of epinephrine from USP Epinephrine Bitartrate RS and 20  $\mu$ g/mL of norepinephrine from USP Norepinephrine Bitartrate RS in *Diluent*

**Standard stock solution:** 0.55 mg/mL of epinephrine from USP Epinephrine Bitartrate RS in *Diluent*

**Standard solution:** Dilute a suitable volume of the *Standard stock solution* with *Diluent* to obtain a final concentration of  $L$  mg/mL of epinephrine, where  $L$  is the label claim of epinephrine in the Injection.

**Sample solution:** Use the Injection directly.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Amperometric electrochemical

**Reference electrode:** Silver/silver chloride

**Working electrode:** Glassy carbon

**Potential:** +650 mV

**Detector temperature:**  $28 \pm 2^\circ$

**Column:** 4.0-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Flow rate:** 1 mL/min

**Injection size:** 2  $\mu$ L

**Run time:** 1.7 times the retention time of epinephrine

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for norepinephrine and epinephrine are 0.90 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between the norepinephrine and epinephrine peaks

**Tailing factor:** NMT 2.0 for the epinephrine peak

**Relative standard deviation:** NMT 1.0% for the epinephrine peak, from six injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of epinephrine ( $C_9H_{13}NO_3$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of epinephrine in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of epinephrine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

### PERFORMANCE TESTS

• **DELIVERABLE VOLUME** (698)

For Articaine Hydrochloride and Epinephrine Injection packaged in single-dose containers: Meets the requirements

### IMPURITIES

• **ORGANIC IMPURITIES, LIMIT OF ARTICAINE RELATED COMPOUNDS**

**Mobile phase, Standard stock solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay for Articaine Hydrochloride*.

**Standard solution:** 0.8 mg/mL of USP Articaine Hydrochloride RS from *Standard stock solution* and 40  $\mu$ g/mL of USP Articaine Related Compound B RS in *Mobile phase*



**System suitability****Sample:** *Standard solution*[NOTE—See *Table 1* for relative retention times.]**Suitability requirements****Tailing factor:** NMT 2.2 for the articaïne peak**Resolution:** NLT 1.25 between the articaïne related compound B and articaïne peaks**Relative standard deviation:** NMT 1.0% for the articaïne peak**Analysis****Samples:** *Standard solution* and *Sample solution*

[NOTE—Articaïne related compounds elute at relative retention times of NMT 2.0 with respect to the articaïne peak.]

Calculate the percentage of articaïne related compounds and any other individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = response of each individual impurity from the *Sample solution* $r_S$  = response of articaïne from the *Standard solution* $C_S$  = concentration of articaïne in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of articaïne in the *Sample solution* (mg/mL)

[NOTE—Disregard any peak below 0.05%.]

**Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Articaïne related compound B	0.6	0.5
Articaïne	1.0	—
Any other individual impurity	—	0.2
Total impurities	—	0.5

**• ORGANIC IMPURITIES, LIMIT OF EPINEPHRINE RELATED COMPOUNDS**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay for Epinephrine.

**Analysis****Samples:** *Standard solution* and *Sample solution*

[NOTE—Epinephrine related compounds elute between relative retention times of 0.35 and 1.0, with respect to the epinephrine peak.]

Calculate the percentage of epinephrine related compounds and any other individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = response of each individual impurity from the *Sample solution* $r_S$  = response of epinephrine from the *Standard solution* $C_S$  = concentration of epinephrine in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of epinephrine in the *Sample solution* (mg/mL)**Acceptance criteria:** See *Table 2*.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Epinephrine sulfonate <sup>a</sup>	0.46	7.5
Specified impurity	0.52	8
Epinephrine	1.0	—
Any other individual impurity	—	1
Total impurities	—	10

<sup>a</sup> 1-(3,4-Dihydroxyphenyl)-2-(methylamino)ethanesulfonic acid.**SPECIFIC TESTS****• PH (791):** 2.7–5.2**• BACTERIAL ENDOTOXINS TEST (85):** NMT 0.7 USP Endotoxin Unit/mg of articaïne hydrochloride**• STERILITY TESTS (71):** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.**• PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements**• OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products (1)*.**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass. Store at controlled room temperature.**• USP REFERENCE STANDARDS (11)**

USP Articaïne Hydrochloride RS

USP Articaïne Related Compound B RS

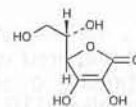
4-Methyl-3-[[2-(propylamino)propanoyl]amino]thiophene-2-carboxylic acid.

C<sub>12</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>S 270.35

USP Endotoxin RS

USP Epinephrine Bitartrate RS

USP Norepinephrine Bitartrate RS

**Ascorbic Acid**C<sub>6</sub>H<sub>8</sub>O<sub>6</sub>

L-Ascorbic acid [50-81-7].

176.12

**DEFINITION**Ascorbic Acid contains NLT 99.0% and NMT 100.5% of C<sub>6</sub>H<sub>8</sub>O<sub>6</sub>.**IDENTIFICATION****• A. INFRARED ABSORPTION (197K)****• B.** A 20-mg/mL solution reduces alkaline cupric tartrate TS slowly at room temperature but more readily upon heating.**ASSAY****• PROCEDURE****Sample:** 400 mg of Ascorbic Acid**Titrimetric system**(See *Titrimetry (541)*.)**Mode:** Direct titration**Titrant:** 0.1 N iodine VS**Endpoint detection:** Visual**Blank:** 100 mL of water and 25 mL of 2 N sulfuric acid. Add 3 mL of starch TS.



**Analysis:** Dissolve the *Sample* in a mixture of 100 mL of water and 25 mL of 2 N sulfuric acid. Add 3 mL of starch TS, and titrate immediately with *Titrant* until a persistent violet-blue color is obtained. Calculate the percentage of ascorbic acid ( $C_6H_8O_6$ ) in the portion of Ascorbic Acid taken:

$$\text{Result} = [(V - B) \times N \times F \times 100] / W$$

*V* = sample titrant volume (mL)  
*B* = blank titrant volume (mL)  
*N* = titrant normality (mEq/mL)  
*F* = equivalency factor, 88.06 mg/mEq  
*W* = weight of *Sample* (mg)  
**Acceptance criteria:** 99.0%–100.5%

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

#### Delete the following:

- **HEAVY METALS** (231)

**Sample solution:** 1 g in 25 mL of water

**Acceptance criteria:** NMT 20 ppm (Official 1-Jan-2018)

#### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation** (781S)  
**Sample solution:** 100 mg/mL in carbon dioxide-free water. Perform the test immediately after preparation of the *Sample solution*.  
**Acceptance criteria:** +20.5° to +21.5°

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Ascorbic Acid RS

## Ascorbic Acid Injection

#### DEFINITION

Ascorbic Acid Injection is a sterile solution, in Water for Injection, of Ascorbic Acid prepared with the aid of Sodium Hydroxide, Sodium Carbonate, or Sodium Bicarbonate. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ).

#### IDENTIFICATION

- **A.**  
**Analysis:** To a volume of Injection, equivalent to 40 mg of ascorbic acid, add 4 mL of 0.1 N hydrochloric acid, then add 4 drops of methylene blue TS, and warm to 40°. **Acceptance criteria:** The deep blue color becomes appreciably lighter or is completely discharged within 3 min.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the Assay.

#### Delete the following:

- **C. IDENTIFICATION TESTS—GENERAL, Sodium** (191): Meets the requirements ▲USP40

#### Add the following:

- **C.** The Injection imparts an intense yellow color to a nonluminous flame. ▲USP40

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Dissolve 15.6 g of dibasic sodium phosphate and 12.2 g of monobasic potassium phosphate in 2000 mL of water, and adjust with phosphoric acid to a pH of  $2.5 \pm 0.05$ .

**Standard solution:** 0.5 mg/mL of USP Ascorbic Acid RS in *Mobile phase*. [NOTE—Refrigerate and store protected from light until use. The solution is stable for at least 24 h. Inject within 3 h after removal from the refrigerator.]

**Sample solution:** Dilute the Injection, if necessary, with *Mobile phase* to obtain a solution with a concentration of about 0.5 mg/mL. [NOTE—Refrigerate and store protected from light until use. The solution is stable for at least 24 h. Inject within 3 h after removal from the refrigerator.]

##### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 245 nm

**Column:** 150-cm  $\times$  6-mm; packing L39

**Flow rate:** 0.6 mL/min

**Injection volume:** 4  $\mu$ L

##### System suitability

**Sample:** *Standard solution*

##### Suitability requirements

**Column efficiency:** NLT 3500 theoretical plates

**Tailing factor:** NMT 1.6

**Relative standard deviation:** NMT 1.5%

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of ascorbic acid in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### IMPURITIES

##### • LIMIT OF OXALATE

**Analysis:** Dilute a volume of Injection, equivalent to 50 mg of ascorbic acid, with water to 5 mL. Add 0.2 mL of acetic acid and 0.5 mL of calcium chloride TS.

**Acceptance criteria:** No turbidity is produced in 1 min.

#### SPECIFIC TESTS

- **PH** (791): 5.5–7.0

- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 1.2 USP Endotoxin Units/mg of ascorbic acid.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in light-resistant, single-dose containers, preferably of Type I or Type II glass.
- **LABELING:** In addition to meeting the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*, fused-seal containers of the Injection in concentrations of 250 mg/mL and greater are labeled to indicate that since pressure may develop on long storage, precautions should be taken to wrap the container in a protective covering while it is being opened.



• **USP REFERENCE STANDARDS** (11)

USP Ascorbic Acid RS  
USP Endotoxin RS

## Ascorbic Acid Oral Solution

### DEFINITION

Ascorbic Acid Oral Solution is a solution of Ascorbic Acid in a hydroxylic organic solvent or an aqueous mixture thereof. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ).

### IDENTIFICATION

• **A.**

**Sample solution:** A volume of Oral Solution equivalent to 40 mg of ascorbic acid

**Analysis:** To the *Sample solution* add 4 mL of 0.1 N hydrochloric acid, then 4 drops of methylene blue TS, and warm to 40°.

**Acceptance criteria:** The deep blue color becomes appreciably lighter or is completely discharged within 3 min.

• **B.**

**Sample solution:** A volume of Oral Solution equivalent to 20 mg of ascorbic acid

**Analysis:** To the *Sample solution* add 15 mL of trichloroacetic acid solution (1 in 20). Add 200 mg of activated charcoal, shake the mixture vigorously for 1 min, and pass through a small fluted filter, returning the filtrate, if necessary, until clear. To 5 mL of the filtrate add 1 drop of pyrrole, agitate gently until dissolved, then heat in a bath at 50°.

**Acceptance criteria:** A blue color develops.

### ASSAY

• **PROCEDURE**

**Sample solution:** Transfer a volume of Oral Solution equivalent to 50 mg of ascorbic acid, previously diluted with water if necessary, to a 100-mL volumetric flask. Add 20 mL of metaphosphoric-acetic acid TS, dilute with water to volume, and mix.

**Blank:** A mixture of 5.5 mL of metaphosphoric-acetic acid TS and 15 mL of water

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** Standard dichlorophenol-indophenol VS

**Analysis:** Transfer a volume of the *Sample solution*, equivalent to 2 mg of ascorbic acid, into a 50-mL conical flask. Add 5 mL of metaphosphoric-acetic acid TS, and titrate with *Titrant* until a rose-pink color persists for at least 5 s. Correct for the volume of the *Titrant* consumed by the *Blank*.

Calculate the percentage of ascorbic acid ( $C_6H_8O_6$ ) in the portion of Oral Solution taken:

$$\text{Result} = \{[(V_S - V_B) \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample solution* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$F$  = concentration of *Titrant* in terms of its equivalent of ascorbic acid (mg/mL)

$W$  = nominal amount of ascorbic acid taken for *Analysis* (mg)

**Acceptance criteria:** 90.0%–110.0%

### OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method I (611)** (if present): 90.0%–110.0% of the labeled content of alcohol ( $C_2H_5OH$ )

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label Oral Solution that contains alcohol to state the alcohol content.

## Ascorbic Acid Tablets

### DEFINITION

Ascorbic Acid Tablets contain ascorbic acid in the form of ascorbic acid ( $C_6H_8O_6$ ), sodium ascorbate ( $C_6H_7NaO_6$ ), calcium ascorbate dihydrate ( $C_{12}H_{14}CaO_{12} \cdot 2H_2O$ ), or their mixture in an amount equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ).

### IDENTIFICATION

• **A.**

**Sample solution:** Triturate a quantity of finely powdered Tablets with diluted alcohol to make a solution of ascorbic acid with a concentration of 20 mg/mL, and filter.

**Analysis:** Add alkaline cupric tartrate TS to a portion of the *Sample solution*.

**Acceptance criteria:** The *Sample solution* reduces alkaline cupric tartrate TS slowly at room temperature but more readily upon heating.

• **B.**

**Sample solution:** Use the *Sample solution* from Identification test A.

**Analysis:** To 2 mL of the *Sample solution* add 4 drops of methylene blue TS, and warm to 40°.

**Acceptance criteria:** The deep blue color of methylene blue becomes appreciably lighter or is completely discharged within 3 min.

• **C.**

**Sample solution:** Use the *Sample solution* from Identification test A.

**Analysis:** To 1 mL of the *Sample solution* add 15 mL of trichloroacetic acid solution (1 in 20) and 200 mg of activated charcoal, shake the mixture vigorously for 1 min, and pass through a small fluted filter, returning the filtrate if necessary, until clear. To 5 mL of the filtrate add 1 drop of pyrrole, agitate gently until dissolved, and then heat in a bath at 50°.

**Acceptance criteria:** A blue color develops.

### ASSAY

[NOTE—Where more than one assay procedure is given in the monograph, the requirements may be met by following any one of the specified procedures, the procedure used being stated in the labeling only if *Procedure 1* is not used.]

• **PROCEDURE 1**

**Sample stock solution:** Transfer NLT 20 Tablets to a 1000-mL volumetric flask containing 250 mL of metaphosphoric-acetic acids TS. Insert the stopper in the flask, and shake by mechanical means for 30 min or until the Tablets have disintegrated completely. Dilute with water to volume.

**Sample solution:** Transfer a portion of the *Sample stock solution* to a centrifuge tube, and centrifuge until a clear supernatant is obtained. Quantitatively dilute the clear supernatant with water, if necessary, to obtain a solution containing 0.5 mg/mL of ascorbic acid.

**Blank:** A mixture of 5.5 mL of metaphosphoric-acetic acids TS and 15 mL of water

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** Standard dichlorophenol-indophenol VS

**Endpoint detection:** Visual, a rose-pink color that persists for at least 5 s



**Analysis:** Transfer a volume of the *Sample solution*, equivalent to 2 mg of ascorbic acid, to a 50-mL conical flask. Add 5 mL of metaphosphoric-acetic acids TS, and titrate with *Titrant*. Correct for the volume of the *Titrant* consumed by the *Blank*.

Calculate the percentage of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ) in the portion of Tablets taken:

$$\text{Result} = [(V_S - V_B) \times F/W] \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample solution* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$F$  = concentration of the *Titrant* in terms of the equivalent of ascorbic acid (mg/mL)

$W$  = nominal weight of ascorbic acid taken for *Analysis* (mg)

**Acceptance criteria:** 90.0%–110.0%

#### PROCEDURE 2

(See *Vitamin C Assay* (580), *Method II—Chromatographic Method*.)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Sample solution:** Withdraw a portion of the solution under test, pass through a suitable filter, and use the pooled sample as the test specimen.

**Analysis:** Proceed as directed in the *Assay, Procedure 1* or *Procedure 2*, conducting the procedure without delay and making any necessary modifications.

Calculate the percentage of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ) dissolved:

For *Procedure 1*

$$\text{Result} = [(V_S - V_B) \times F \times (V_M/a)/L] \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample solution* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$F$  = concentration of the *Titrant* in terms of the equivalent of ascorbic acid (mg/mL)

$V_M$  = volume of *Medium*, 900 mL

$a$  = volume of the aliquot taken for *Analysis*

$L$  = label claim of ascorbic acid (mg/Tablet)

For *Procedure 2*

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak area of ascorbic acid from the *Sample solution*

$r_S$  = peak area of ascorbic acid from the *Standard solution*

$C_S$  = concentration of USP Ascorbic Acid RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of ascorbic acid ( $C_6H_8O_6$ ) is dissolved.

##### DISINTEGRATION (701)

[NOTE—Meet this additional test if the label recommends to disintegrate the Tablets in the mouth before swallowing.]

**Medium:** Water

**Time:** NMT 5 min

**Acceptance criteria:** Meet the requirements

##### UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

#### ADDITIONAL REQUIREMENTS

##### PACKAGING AND STORAGE:

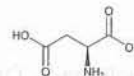
Preserve in tight, light-resistant containers.

- LABELING:** The label states the quantity of ascorbic acid in mg/Tablet, and the chemical form of ascorbic acid present in the Tablets. The labeling states with which assay procedure the product complies only if *Procedure 1* is not used. Tablets that are intended to be disintegrated in the mouth before swallowing are so labeled.

##### USP REFERENCE STANDARDS (11)

USP Ascorbic Acid RS

## Aspartic Acid



$C_4H_7NO_4$

133.10

L-Aspartic acid [56-84-8].

#### DEFINITION

Aspartic Acid contains NLT 98.5% and NMT 101.5% of aspartic acid ( $C_4H_7NO_4$ ), calculated on the dried basis.

#### IDENTIFICATION

##### A. INFRARED ABSORPTION (197K)

#### ASSAY

##### PROCEDURE

**Sample:** 100 mg of Aspartic Acid

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N sodium hydroxide VS

**Endpoint detection:** Visual

**Blank:** 50 mL of carbon dioxide-free water. Add 0.1 mL of bromothymol blue TS.

**Analysis:** Transfer the *Sample* to a 125-mL flask, and dissolve in 50 mL of carbon dioxide-free water. Heat slightly if necessary. Cool, add 0.1 mL of bromothymol blue TS, and titrate with *Titrant* until the color changes from yellow to blue. Perform the blank determination. Calculate the percentage of aspartic acid ( $C_4H_7NO_4$ ) in the portion of Aspartic Acid taken:

$$\text{Result} = [(V - B) \times N \times F \times 100]/W$$

$V$  = *Titrant* volume consumed by the *Sample* (mL)

$B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 133.1 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 98.5%–101.5% on the dried basis

#### IMPURITIES

##### RESIDUE ON IGNITION (281):

NMT 0.1%

##### CHLORIDE AND SULFATE, Chloride (221)

**Sample solution:** Dissolve 0.7 g of Aspartic Acid in 10 mL of diluted nitric acid, and dilute with water to 15 mL.

**Acceptance criteria:** The *Sample solution* shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid (NMT 0.02%).

##### CHLORIDE AND SULFATE, Sulfate (221)

**Sample solution:** Dissolve 0.8 g of Aspartic Acid in 4 mL of hydrochloric acid, and dilute with water to 15 mL.

**Acceptance criteria:** The *Sample solution* shows no more sulfate than corresponds to 0.25 mL of 0.020 N sulfuric acid (NMT 0.03%).



- **IRON** (241): NMT 10 ppm

**Delete the following:**

- **HEAVY METALS, Method II** (231): NMT 10 ppm • (Official 1-

Jan-2018)

- **RELATED COMPOUNDS**

**Mobile phase:** 0.008 N sulfuric acid

**System suitability solution:** A mixture of 0.1 mg/mL of USP Fumaric Acid RS, 0.05 mg/mL of USP Maleic Acid RS, and 1.5 mg/mL of USP Malic Acid RS in water

**Fumaric acid standard solution:** 0.1 mg/mL of USP Fumaric Acid RS in water

**Maleic acid standard solution:** 0.05 mg/mL of USP Maleic Acid RS in water

**Malic acid standard solution:** 1.5 mg/mL of USP Malic Acid RS in water

**Sample solution:** Transfer 10 g of Aspartic Acid into a 100-mL volumetric flask, add 50 mL of water and, if necessary, a few drops of 6 N hydrochloric acid to help dissolve the sample. Dilute with water to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 7.8-mm × 30-cm; 9-μm packing L17

**Column temperature:** 30°

**Flow rate:** 0.6 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between maleic acid and malic acid

**Relative standard deviation:** NMT 10.0% each for maleic acid, malic acid, and fumaric acid

**Analysis**

**Samples:** Standard solutions and *Sample solution*

Calculate the percentage of each specified acid in the portion of Aspartic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of maleic acid, malic acid, or fumaric acid from the *Sample solution*

$r_S$  = peak response of maleic acid, malic acid, or fumaric acid from the corresponding Standard solution

$C_S$  = concentration of USP Maleic Acid RS, USP Malic Acid RS, or USP Fumaric Acid RS in the corresponding Standard solution (mg/mL)

$C_U$  = concentration of Aspartic Acid in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Aspartic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity from the *Sample solution*

$r_S$  = peak response of fumaric acid from the *Fumaric acid standard solution*

$C_S$  = concentration of USP Fumaric Acid RS in the *Fumaric acid standard solution* (mg/mL)

$C_U$  = concentration of Aspartic Acid in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Maleic acid	0.5	0.05
Malic acid	0.6	0.20
Fumaric acid	1.0	0.10
Aspartic acid	Not observed	—
Any unspecified impurity	—	0.05
Total unspecified impurities	—	0.10

**SPECIFIC TESTS**

- **OPTICAL ROTATION, Specific Rotation** (781S)

**Sample solution:** 80 mg/mL in 6 N hydrochloric acid

**Acceptance criteria:** +24.0° to +26.0°, at 20°

- **LOSS ON DRYING** (731)

**Analysis:** Dry at 105° for 3 h.

**Acceptance criteria:** NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store protected from light.

- **USP REFERENCE STANDARDS** (11)

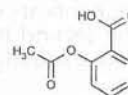
USP Aspartic Acid RS

USP Fumaric Acid RS

USP Maleic Acid RS

USP Malic Acid RS

## Aspirin



$C_9H_8O_4$  180.16

Benzoic acid, 2-(acetyloxy)-.

Salicylic acid acetate [50-78-2].

» Aspirin contains not less than 99.5 percent and not more than 100.5 percent of  $C_9H_8O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Aspirin RS

**Identification**—

**A:** Heat it with water for several minutes, cool, and add 1 or 2 drops of ferric chloride TS: a violet-red color is produced.

**B:** *Infrared Absorption* (197K).

**Loss on drying** (731)—Dry it over silica gel for 5 hours: it loses not more than 0.5% of its weight.

**Readily carbonizable substances** (271)—Dissolve 500 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid Q*.

**Residue on ignition** (281): not more than 0.05%.

**Substances insoluble in sodium carbonate TS**—A solution of 500 mg in 10 mL of warm sodium carbonate TS is clear.

**Chloride** (221)—Boil 1.5 g with 75 mL of water for 5 minutes, cool, add sufficient water to restore the original volume, and filter. A 25-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).



**Sulfate**—Dissolve 6.0 g in 37 mL of acetone, and add 3 mL of water. Titrate potentiometrically with 0.02 M lead perchlorate, prepared by dissolving 9.20 g of lead perchlorate in water to make 1000 mL of solution, using a pH meter capable of a minimum reproducibility of  $\pm 0.1$  mV (see *pH* (791)) and equipped with an electrode system consisting of a lead-specific electrode and a silver-silver chloride reference glass-sleeved electrode containing a solution of tetraethylammonium perchlorate in glacial acetic acid (1 in 44) (see *Titrimetry* (541)); not more than 1.25 mL of 0.02 M lead perchlorate is consumed (0.04%). [NOTE—After use, rinse the lead-specific electrode with water, drain the reference electrode, flush with water, rinse with methanol, and allow to dry.]

#### Delete the following:

**•Heavy metals**—Dissolve 2 g in 25 mL of acetone, and add 1 mL of water. Add 1.2 mL of thioacetamide-glycerin base TS and 2 mL of pH 3.5 Acetate Buffer (see *Heavy Metals* (231)), and allow to stand for 5 minutes: any color produced is not darker than that of a control made with 25 mL of acetone and 2 mL of *Standard Lead Solution* (see *Heavy Metals* (231)), treated in the same manner. The limit is 10  $\mu$ g per g. • (Official 1-Jan-2018)

**Limit of free salicylic acid**—Dissolve 2.5 g in sufficient alcohol to make 25.0 mL. To each of two matched color-comparison tubes add 48 mL of water and 1 mL of a freshly prepared, diluted ferric ammonium sulfate solution (prepared by adding 1 mL of 1 N hydrochloric acid to 2 mL of ferric ammonium sulfate TS and diluting with water to 100 mL). Into one tube pipet 1 mL of a standard solution of salicylic acid in water, containing 0.10 mg of salicylic acid per mL. Into the second tube pipet 1 mL of the 1 in 10 solution of Aspirin. Mix the contents of each tube: after 30 seconds, the color in the second tube is not more intense than that in the tube containing the salicylic acid (0.1%).

**Assay**—Place about 1.5 g of Aspirin, accurately weighed, in a flask, add 50.0 mL of 0.5 N sodium hydroxide VS, and boil the mixture gently for 10 minutes. Add phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 N sulfuric acid VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.5 N sodium hydroxide is equivalent to 45.04 mg of  $C_9H_8O_4$ .

## Aspirin Boluses

» Aspirin Boluses contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label Boluses to indicate that they are for veterinary use only.

#### USP Reference standards (11)—

USP Aspirin RS

USP Salicylic Acid RS

#### Identification—

**A:** Crush 1 Bolus, boil a portion of the powder, equivalent to about 300 mg of aspirin, with 50 mL of water, cool, and add a drop of ferric chloride TS: a violet-red color is produced.

**B:** The retention time of the aspirin peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Dissolution (711)—

*Medium:* 0.5 M phosphate buffer, pH 7.4; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 45 minutes.

*Diluting solution*—Prepare a mixture of acetonitrile and formic acid (99:1).

*Procedure*—Determine the amount of aspirin ( $C_9H_8O_4$ ) dissolved by employing UV absorption at the wavelength of the isosbestic point of aspirin and salicylic acid at  $265 \pm 2$  nm on filtered portions of the solution under test, suitably diluted with *Diluting solution*, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same *Medium*. [NOTE—Prepare the Standard solution at the time of use.]

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_9H_8O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of salicylic acid**—Using the chromatograms of the *Standard preparation* and the *Assay preparation*, obtained as directed in the *Assay*, calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Boluses taken by the formula:

$$100,000(C/W_A)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*;  $W_A$  is the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Boluses taken, as determined in the *Assay*; and  $r_U$  and  $r_S$  are the salicylic acid peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 0.3% is found.

#### Assay—

*Mobile phase*—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4. Make any necessary adjustments (see *System Suitability* under *Chromatography* (621)).

*Diluting solution*—Prepare a mixture of acetonitrile and formic acid (99:1).

*Standard preparation*—Prepare a solution in *Diluting solution* having known concentrations of about 0.4 mg of USP Aspirin RS and 0.01 mg of USP Salicylic Acid RS per mL.

*Assay preparation*—Weigh and finely powder not fewer than 10 Boluses. Transfer an accurately weighed portion of the powder, equivalent to about 400 mg of aspirin, to a 100-mL volumetric flask, dilute with *Diluting solution* to volume, and stir by mechanical means for about 15 minutes. Pass a portion of this solution through a filter having a 0.5- $\mu$ m or finer porosity, and use the filtrate as the *Assay preparation*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for salicylic acid and 1.0 for aspirin, and the relative standard deviation of the aspirin peak response for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Boluses taken by the formula:

$$1000C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the



aspirin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aspirin Capsules

» Aspirin Capsules contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

NOTE—Capsules that are enteric-coated or the contents of which are enteric-coated meet the requirements for *Aspirin Delayed-Release Capsules*.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Aspirin RS

**Identification**—

**A:** Heat about 100 mg of the Capsule contents with 10 mL of water for several minutes, cool, and add 1 drop of ferric chloride TS: a violet-red color is produced.

**B:** Shake a quantity of the contents of Capsules, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant and evaporate it to dryness. Dry the residue in vacuum at 60° for 1 hour: the residue responds to *Identification test B* under *Aspirin*.

**Dissolution** (711)—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 500 mL.

**Apparatus 1:** 100 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_9H_8O_4$  dissolved from UV absorbances at the wavelength of the isosbestic point of aspirin and salicylic acid at  $265 \pm 2$  nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same *Medium*. [NOTE—Prepare the Standard solution at the time of use. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to bring the Reference Standard into solution prior to dilution with *Medium*.]

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_9H_8O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of free salicylic acid**—

**Ferric chloride-urea reagent**—Dissolve by swirling, without the aid of heat, 60 g of urea in a mixture of 8 mL of ferric chloride solution (6 in 10) and 42 mL of 0.05 N hydrochloric acid. Adjust the resulting solution, if necessary, with 6 N hydrochloric acid to a pH of 3.2.

**Standard preparation**—Transfer 75.0 mg of salicylic acid, previously dried over silica gel for 3 hours and accurately weighed, to a 100-mL volumetric flask, add chloroform to volume, and mix. Transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with chloroform to volume, and mix. Transfer 10.0 mL of this last solution to a 50-mL volumetric flask containing 10 mL of methanol, 2 drops of hydrochloric acid, and 10 mL of a 1 in 10 solution of glacial acetic acid in ether, dilute with chloroform to volume, and mix.

**Chromatographic column** (see *Chromatography* (621))—Proceed as directed under *Column Partition Chromatography*, packing a chromatographic tube with two segments of packing material. The lower segment is a mixture of 1 g of *Solid Support* and 0.5 mL of 5 M phosphoric acid, and the upper segment is a mixture of 3 g of *Solid Support* and 2 mL of freshly prepared *Ferric chloride-urea reagent*.

**Test preparation**—Weigh accurately a portion of the contents of the Capsules, as determined by the *Assay*, equivalent to 100 mg of aspirin, mix with 10 mL of chloroform by stirring for 3 minutes, and then transfer to the chromatographic column with the aid of a few mL of chloroform. Pass 50 mL of chloroform through the column, rinse the tip of the chromatographic tube with chloroform, and discard the eluate. Prepare as a receiver a 50-mL volumetric flask containing 10 mL of methanol and 2 drops of hydrochloric acid, and elute any salicylic acid from the column by passing 10 mL of a 1 in 10 solution of glacial acetic acid in ether that has been recently saturated with water, followed by 30 mL of chloroform. Dilute the eluate with chloroform to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 306 nm, with a suitable spectrophotometer, using as the blank a solvent mixture of the same composition as that used for the *Standard preparation*: the absorbance of the *Test preparation* does not exceed that of the *Standard preparation* (0.75%, calculated on the labeled aspirin content).

**Assay**—[NOTE—In this assay use chloroform recently saturated with water.]

**Standard preparation**—Transfer about 50 mg of USP Aspirin RS, accurately weighed, to a 50-mL volumetric flask, add 0.5 mL of glacial acetic acid, add chloroform to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with a 1 in 100 solution of glacial acetic acid in chloroform to volume, and mix. The concentration of USP Aspirin RS is about 50 µg per mL.

**Chromatographic column**—Proceed as directed under *Column Partition Chromatography* (see *Chromatography* (621)), packing a chromatographic tube with a mixture of 3 g of *Solid Support* and 2 mL of freshly prepared sodium bicarbonate solution (1 in 12).

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to about 50 mg of aspirin, to a 50-mL volumetric flask containing 1 mL of a 1 in 50 solution of hydrochloric acid in methanol, add chloroform to volume, and mix. Transfer 5.0 mL of this solution to the column, wash with 5 mL and then with 25 mL of chloroform, and discard the washings. Elute into a 100-mL volumetric flask with about 10 mL of a 1 in 10 solution of glacial acetic acid in chloroform and then with about 85 mL of a 1 in 100 solution of glacial acetic acid in chloroform, dilute with the latter solvent to volume, and mix.

**Procedure**—Without delay, concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Capsules taken by the formula:

$$C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Aspirin RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.



## Aspirin Delayed-Release Capsules

» Aspirin Delayed-Release Capsules contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The label indicates that the Capsules or the contents thereof are enteric-coated.

**USP Reference standards** (11)—

USP Aspirin RS

USP Salicylic Acid RS

**Identification**—

**A:** Heat about 100 mg of the Capsule contents with 10 mL of water for several minutes, cool, and add 1 drop of ferric chloride TS: a violet-red color is produced.

**B: Infrared Absorption** (197K)—Prepare the test specimen as follows. Shake a quantity of the contents of Capsules, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant and evaporate it to dryness. Dry the residue in vacuum at 60° for 1 hour.

**Dissolution** (711)—Proceed as directed for *Procedure* for *Method A* under *Apparatus 1* and *Apparatus 2*, *Delayed-Release Dosage Forms*.

*Apparatus 1:* 100 rpm.

*Time:* 90 minutes, for *Buffer stage*.

**Diluent**—Prepare a mixture of 0.1 N hydrochloric acid and 0.20 M tribasic sodium phosphate (3:1), and adjust, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of  $6.8 \pm 0.05$ .

**Procedure**—Determine the amount of  $C_9H_8O_4$  dissolved by determining UV absorbances at the wavelength of the isobestic point of aspirin and salicylic acid (about 280 nm in the *Acid stage*, and about 265 nm in the *Buffer stage*), using a filtered portion of the solution under test, diluted, if necessary, with 0.1 N hydrochloric acid (analyzing the *Acid stage*) and with *Diluent* (analyzing the *Buffer stage*), in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same medium.

**Uniformity of dosage units** (905): meet the requirements.

**Limit of free salicylic acid**—

**Mobile phase and Diluting solution**—Prepare as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in the *Standard preparation*, prepared as directed in the *Assay*, to obtain a solution having a known concentration of about 0.015 mg of salicylic acid per mL.

**Test solution**—Use the *Stock solution* prepared as directed for *Assay preparation*.

**Chromatographic system**—Use the *Chromatographic system* described in the *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure* in the *Assay*: the relative retention times are about 0.7 for salicylic acid and 1.0 for aspirin; the resolution, *R*, between salicylic acid and aspirin is not less than 2.0; and the relative standard deviation of the salicylic acid peak responses is not more than 4.0%.

**Procedure**—Proceed as directed for *Procedure* in the *Assay*. Calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Capsules taken by the formula:

$$2000(C / Q_A)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard solution*; *Q<sub>A</sub>* is the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Capsules taken, as determined in the *Assay*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses of the salicylic acid peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% is found.

**Assay**—

**Mobile phase**—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4.

**Diluting solution**—Prepare a mixture of acetonitrile and formic acid (99:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Aspirin RS in *Diluting solution* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of aspirin, to a suitable container. Add 20.0 mL of *Diluting solution* and about 10 glass beads. Shake vigorously for about 10 minutes, and centrifuge (*Stock solution*). Quantitatively dilute an accurately measured volume of the *Stock solution* with 9 volumes of *Diluting solution* (*Assay preparation*). Retain the remaining portion of *Stock solution* for the test for *Limit of free salicylic acid*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not greater than 2.0; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Capsules taken by the formula:

$$200C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses of the aspirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aspirin Suppositories

» Aspirin Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in well-closed containers, in a cool place.

**USP Reference standards** (11)—

USP Aspirin RS

**Identification**—Transfer a portion of the melted Suppositories obtained in the *Assay*, equivalent to about 1 g of aspirin, to a 125-mL conical flask. Add 20 mL of alcohol, and



warm until completely disintegrated. Cool in an ice bath for 5 minutes, filter, and evaporate the filtrate to dryness: the residue responds to *Identification* tests A and B under *Aspirin*.

#### Limit of free salicylic acid—

**Ferric chloride-urea reagent**—To a mixture of 8 mL of ferric chloride solution (6 in 10) and 42 mL of 0.05 N hydrochloric acid add 60 g of urea. Dissolve the urea by swirling and without the aid of heat, and adjust the resulting solution, if necessary, by the addition of 6 N hydrochloric acid to a pH of 3.2. Prepare on the day of use.

**Procedure**—Insert a small pledget of glass wool above the stem constriction of a 20- × 2.5-cm chromatographic tube, and uniformly pack with a mixture of about 1 g of chromatographic siliceous earth and 0.5 mL of 5 M phosphoric acid. Directly above this layer, pack a similar mixture of about 3 g of chromatographic siliceous earth and 2 mL of *Ferric chloride-urea reagent*. Transfer to a small beaker an accurately weighed portion of the cooled mass from the previously melted Suppositories obtained in the *Assay*, equivalent to 50 mg of aspirin, add 10 mL of chloroform, warm slightly, and stir until dissolved. With the aid of 5 mL of chloroform, transfer the mixture to the chromatographic adsorption column. Pass 50 mL of chloroform in several portions through the column, rinse the tip of the chromatographic tube with chloroform, and discard the eluate. If the purple zone reaches the bottom of the tube, discard the column, and repeat the test with a smaller quantity of melted Suppositories.

Elute the adsorbed salicylic acid into a 100-mL volumetric flask containing 20 mL of methanol and 0.2 mL of hydrochloric acid by passing two 10-mL portions of a 1 in 10 solution of glacial acetic acid in water-saturated ether, and then 30 mL of chloroform, through the column, and dilute the eluate with chloroform to volume. Dissolve a suitable, accurately weighed quantity of salicylic acid in chloroform to obtain a Standard solution containing 150 µg of salicylic acid per mL. Pipet 5 mL of this solution into a 50-mL volumetric flask containing 10 mL of methanol, 0.1 mL of hydrochloric acid, and 10 mL of a 1 in 10 solution of glacial acetic acid in ether. Add chloroform to volume, and mix. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 306 nm, using as the blank a solvent mixture of the same composition as that of the Standard solution: the absorbance of the solution from the Suppositories does not exceed that of the Standard solution (3.0%).

**Assay**—[NOTE—In this assay, use chloroform that recently was saturated with water.]

**Chromatographic column**—Uniformly pack a chromatographic tube, as described in the test for *Limit of free salicylic acid* for *Procedure*, with a mixture of about 3 g of chromatographic siliceous earth and 2 mL of sodium bicarbonate solution (1 in 12) prepared on the day of use.

**Standard preparation**—Transfer about 50 mg of USP Aspirin RS, accurately weighed, to a 50-mL volumetric flask, add 0.5 mL of glacial acetic acid, and add chloroform to volume. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add a 1 in 100 solution of glacial acetic acid in chloroform to volume, and mix.

**Assay preparation**—Tare a small dish and glass rod, place in the dish not fewer than 5 Suppositories, heat gently on a steam bath until melted, then stir, cool while stirring, and weigh. Transfer an accurately weighed portion of the mass, equivalent to about 50 mg of aspirin, to a 50-mL volumetric flask containing 1 mL of a 1 in 50 solution of hydrochloric acid in methanol, add 40 mL of chloroform, mix, and add chloroform to volume.

**Procedure**—Pipet 5 mL of the *Assay preparation* into the column, wash with 5 mL and then with 25 mL of chloroform, and discard the washings. Without delay, elute into a 100-mL volumetric flask with about 10 mL of a 1 in 10 solution of glacial acetic acid in chloroform, and then with about 85 mL of a 1 in 100 solution of glacial acetic acid in

chloroform, dilute with the latter solvent to volume, and mix. Without delay, concomitantly determine the absorbances of the eluted *Assay preparation* and the *Standard preparation* in 1-cm cells at the wavelength of maximum absorbance at about 280 nm, with a suitable spectrophotometer, using chloroform as the blank. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Suppositories taken by the formula:

$$C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Aspirin RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Aspirin Tablets

» Aspirin Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ). Tablets of larger than 81-mg size contain no sweeteners or other flavors.

NOTE—Tablets that are enteric-coated meet the requirements for *Aspirin Delayed-Release Tablets*.

**Packaging and storage**—Preserve in tight containers. Preserve flavored or sweetened Tablets of 81-mg size or smaller in containers holding not more than 36 Tablets each.

#### USP Reference standards (11)—

USP Aspirin RS

USP Salicylic Acid RS

#### Identification—

**A:** Crush 1 Tablet, boil it with 50 mL of water for 5 minutes, cool, and add 1 or 2 drops of ferric chloride TS: a violet-red color is produced.

**B: Infrared Absorption** (197K)—Prepare the test specimen as follows. Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness. Dry the residue in vacuum at 60° for 1 hour.

#### Dissolution (711)—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 500 mL.

**Apparatus 1:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of  $C_9H_8O_4$  dissolved from UV absorbances at the wavelength of the isosbestic point of aspirin and salicylic acid at  $265 \pm 2$  nm of filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same *Medium*. [NOTE—Prepare the Standard solution at the time of use. An amount of alcohol not to exceed 1% of the total volume of the Standard solution may be used to bring the Reference Standard into solution prior to dilution with *Medium*.]

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_9H_8O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Limit of free salicylic acid—

**Mobile phase and Diluting solution**—Prepare as directed in the *Assay*.



**Standard solution**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in the *Standard preparation* prepared as directed in the *Assay*, to obtain a solution having a known concentration of about 0.015 mg of salicylic acid per mL.

**Test solution**—Use the *Stock solution* prepared as directed for *Assay preparation* in the *Assay*.

**Chromatographic system**—Use the *Chromatographic system* described in the *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for salicylic acid and 1.0 for aspirin; the resolution,  $R$ , between salicylic acid and aspirin is not less than 2.0; and the relative standard deviation of the salicylic acid peak responses is not more than 4.0%.

**Procedure**—Proceed as directed for *Procedure* in the *Assay*. Calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Tablets taken by the formula:

$$2000(C / Q_A)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard solution*;  $Q_A$  is the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken, as determined in the *Assay*, and  $r_U$  and  $r_S$  are the peak responses of the salicylic acid peaks obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.3% is found. In the case of Tablets that are coated, not more than 3.0% is found.

#### Assay—

**Mobile phase**—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4.

**Diluting solution**—Prepare a mixture of acetonitrile and formic acid (99:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Aspirin RS in *Diluting solution* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of aspirin, to a suitable container. Add 20.0 mL of *Diluting solution* and about 10 beads. Shake vigorously for about 10 minutes, and centrifuge (*Stock solution*). Quantitatively dilute an accurately measured volume of the *Stock solution* with 9 volumes of *Diluting solution* (*Assay preparation*). Retain the remaining portion of *Stock solution* for the test for *Limit of free salicylic acid*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm  $\times$  30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not greater than 2.0; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the aspirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Buffered Aspirin Tablets

» Buffered Aspirin Tablets contain Aspirin and suitable buffering agents. Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Aspirin RS

USP Salicylic Acid RS

#### Identification—

**A:** Crush 1 Tablet, boil it with 50 mL of water for 5 minutes, cool, and add 1 or 2 drops of ferric chloride TS: a violet-red color is produced.

**B:** *Infrared Absorption* (197K)—

**Test specimen**—Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of aspirin, with 10 mL of chloroform for several minutes. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness.

#### Dissolution (711)—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 500 mL.

**Apparatus 2:** 75 rpm. [NOTE—Where the Tablet is composed of multiple layers, a stainless steel wire helix may be used, if needed, to hold the Tablet in proper orientation in the apparatus.]

**Time:** 30 minutes.

**Procedure**—Determine the amount of aspirin ( $C_9H_8O_4$ ) dissolved by employing UV absorption at the wavelength of the isosbestic point of aspirin and salicylic acid at  $265 \pm 2$  nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Aspirin RS in the same *Medium*. [NOTE—Prepare the *Standard solution* at the time of use. An amount of methanol not to exceed 1% of the total volume of the *Standard solution* may be used to dissolve the Reference Standard prior to dilution with *Medium*.]

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_9H_8O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Acid-neutralizing capacity** (301): not less than 1.9 mEq of acid is consumed for each 325 mg of aspirin in the Tablets.

#### Limit of free salicylic acid—

**Mobile phase and Diluting solution**—Prepare as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in the *Standard preparation* prepared as directed in the *Assay*, to obtain a solution having a known concentration of about 0.015 mg of salicylic acid per mL.

**Test solution**—Use the *Stock solution*, prepared as directed for *Assay preparation* in the *Assay*.

**Chromatographic system**—Prepare as directed in the *Assay*. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for salicylic acid and 1.0 for aspirin; the resolution,  $R$ , between salicylic acid and aspirin is not less than 2.0; and the relative standard deviation determined from salicylic acid is not more than 4.0%.



**Procedure**—Proceed as directed in the Assay. Calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Tablets taken by the formula:

$$2000(C / Q_A)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard solution*;  $Q_A$  is the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken, as determined in the Assay; and  $r_U$  and  $r_S$  are the peak responses of salicylic acid obtained from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% is found.

#### Assay—

**Mobile phase**—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4.

**Diluting solution**—Prepare a mixture of acetonitrile and formic acid (99:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Aspirin RS in *Diluting solution* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of aspirin, to a suitable container. Add 20.0 mL of *Diluting solution* and about 10 glass beads. Shake vigorously for about 10 minutes, and centrifuge (*Stock solution*). Quantitatively dilute an accurately measured volume of the *Stock solution* with 9 volumes of *Diluting solution* (*Assay preparation*). Retain the remaining portion of *Stock solution* for the test for Limit of free salicylic acid.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of aspirin obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Aspirin Delayed-Release Tablets

### DEFINITION

Aspirin Delayed-Release Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of aspirin ( $C_9H_8O_4$ ).

### IDENTIFICATION

#### • A. PROCEDURE

**Sample:** 1 Tablet

**Analysis:** Crush and boil the *Sample* with 50 mL of water for 5 min, cool, and add 1 or 2 drops of ferric chloride TS.

**Acceptance criteria:** A violet-red color is produced.

#### • B. INFRARED ABSORPTION (197K)

**Sample:** Shake a quantity of finely powdered Tablets, equivalent to 500 mg of aspirin, with 10 mL of alcohol

for several min. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness. Dry the residue under vacuum at 60° for 1 h.

**Acceptance criteria:** Meet the requirements

### ASSAY

#### • PROCEDURE

**Mobile phase:** 2 g/L of sodium 1-heptanesulfonate in acetonitrile and water (15:85). Adjust with glacial acetic acid to a pH of 3.4.

**Diluent:** Acetonitrile and formic acid (99:1)

**Standard solution:** 0.5 mg/mL of USP Aspirin RS in *Diluent*

**Sample stock solution:** Transfer an equivalent to 100 mg of aspirin, from NLT 20 finely powdered Tablets, to a suitable container. Add 20.0 mL of *Diluent* and 10 glass beads. Shake vigorously for 10 min, and centrifuge.

**Sample solution:** Dilute a volume of the *Sample stock solution* with 9 volumes of *Diluent*. [NOTE—Retain the remaining portion of stock solution for the test for Limit of Free Salicylic Acid.]

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.0-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aspirin in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0%

### PERFORMANCE TESTS

• **DISSOLUTION (711):** Proceed as directed for *Procedure* for Method B in *Procedure, Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms*.

**Apparatus 1:** 100 rpm

#### Times

**Acid stage:** 2 h

**Buffer stage:** 90 min

**Diluent:** 0.1 N hydrochloric acid and 0.20 M tribasic sodium phosphate (3:1). Adjust, if necessary, with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of  $6.8 \pm 0.05$ .

**Standard solution:** USP Aspirin RS of a known concentration in *Medium*

**Sample solution:** Filtered portion of the solution under test, diluted, if necessary, with 0.1 N hydrochloric acid (analyzing the *Acid stage*) and with *Diluent* (analyzing the *Buffer stage*)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the quantity of aspirin ( $C_9H_8O_4$ ) dissolved by determining UV absorbances at the wavelength of the isosbestic point of aspirin and salicylic acid (about 280 nm in the *Acid stage*, and about 265 nm in the *Buffer stage*) of the *Sample solution* in comparison to the *Standard solution*.



**Tolerances**

**Acid stage:** NMT 10% (Q) of the labeled amount of aspirin ( $C_9H_8O_4$ ) is dissolved.

**Buffer stage:** NLT 75% (Q) of the labeled amount of aspirin ( $C_9H_8O_4$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**IMPURITIES**• **LIMIT OF FREE SALICYLIC ACID**

Mobile phase, Diluent, and Chromatographic system: Prepare as directed in the Assay.

**Standard solution:** 0.015 mg/mL of USP Salicylic Acid RS and 0.5 mg/mL of USP Aspirin RS in Diluent

**Sample solution:** Use the Sample stock solution from the Assay.

**System suitability**

**Sample:** Standard solution

[NOTE—The relative retention times for salicylic acid and aspirin are about 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between salicylic acid and aspirin

**Relative standard deviation:** NMT 4.0% of the salicylic acid peak responses

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of salicylic acid from the Sample solution

$r_s$  = peak response of salicylic acid from the Standard solution

$C_s$  = concentration of USP Salicylic Acid RS in the Standard solution (mg/mL)

$C_u$  = concentration of aspirin in the Sample solution as determined in the Assay (mg/mL)

**Acceptance criteria:** NMT 3.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Aspirin RS

USP Salicylic Acid RS

**Aspirin Effervescent Tablets for Oral Solution**

» Aspirin Effervescent Tablets for Oral Solution contain Aspirin and an effervescent mixture of a suitable organic acid and an alkali metal bicarbonate and/or carbonate. Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Aspirin RS

USP Salicylic Acid RS

**Identification**—

**A:** Dissolve 1 Tablet in about 50 mL of 1 N hydrochloric acid, boil for about 5 minutes, and allow to cool. To 2 mL of the resulting solution add 2 or 3 drops of ferric chloride TS: a violet-red color is produced.

**B:** Add about one-half a Tablet to 50 mL of water in a flask, and immediately stopper with a stopper fitted with

tubing so that the evolved gas passes through calcium hydroxide TS: a white precipitate forms.

**Solution time**—Two Tablets dissolve completely in 180 mL of water at  $17.5 \pm 2.5^\circ$  within 5 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Acid-neutralizing capacity** (301): not less than 5.0 mEq of acid is consumed by 1 Tablet.

**Limit of free salicylate**—Proceed as directed for Limit of free salicylic acid under Buffered Aspirin Tablets: not more than 8.0% is found.

**Assay**—Proceed as directed in the Assay under Buffered Aspirin Tablets.

**Aspirin Extended-Release Tablets**

» Aspirin Extended-Release Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The labeling indicates the Dissolution Test with which the product complies.

**USP Reference standards** (11)—

USP Aspirin RS

USP Salicylic Acid RS

**Identification**—

**A:** Crush 1 Tablet, boil it with 50 mL of water for 5 minutes, cool, and add 1 or 2 drops of ferric chloride TS: a violet-red color is produced.

**B: Infrared Absorption** (197K)—Prepare the test specimen as follows. Shake a quantity of finely powdered Tablets, equivalent to about 500 mg of aspirin, with 10 mL of alcohol for several minutes. Centrifuge the mixture. Pour off the clear supernatant, and evaporate it to dryness. Dry the residue in vacuum at  $60^\circ$  for 1 hour.

**Dissolution** (711)—

**TEST 1**—If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 1.

**Medium:** 0.1 N hydrochloric acid; 900 mL.

**Apparatus 2:** 60 rpm.

**Times:** 1 hour and 4 hours.

**Procedure**—Determine the amount of  $C_9H_8O_4$  dissolved from UV absorbances at the isosbestic point at about 280 nm, using filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same medium.

**Tolerances**—The percentages of the labeled amount of  $C_9H_8O_4$  dissolved at the times specified conform to Acceptance Table 2.

Time (hours)	Amount dissolved
1	between 20% and 55%
4	not less than 80%

**TEST 2**—If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

**Medium:** water; 1000 mL.

**Apparatus 2:** 30 rpm.

**Times:** 1, 2, 4, and 8 hours.

**Procedure**—Determine the amount of  $C_9H_8O_4$  dissolved from UV absorbances at the isosbestic point at about 265 nm, using filtered portions of the solution under test, suitably



bly diluted with water, if necessary, in comparison with a Standard solution having a known concentration of USP Aspirin RS in the same Medium. [NOTE—Prepare the Standard solution at the time of use. An amount of alcohol not to exceed 5% of the total volume of the Standard solution may be used to bring the USP Reference Standard into solution prior to dilution with Medium.]

**Tolerances**—The percentages of the labeled amount of  $C_9H_8O_4$  dissolved at the times specified conform to Acceptance Table 2.

Time (hours)	Amount dissolved
1	between 15% and 40%
2	between 25% and 60%
4	between 35% and 75%
8	not less than 70%

**Uniformity of dosage units** (905): meet the requirements.

#### Limit of free salicylic acid—

**Mobile phase and Diluting solution**—Prepare as directed in the Assay.

**Standard solution**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in the Standard preparation prepared as directed in the Assay, to obtain a solution having a known concentration of about 0.015 mg of salicylic acid per mL.

**Test solution**—Use the Stock solution, prepared as directed for Assay preparation in the Assay.

**Chromatographic system**—Use the Chromatographic system described in the Assay. Chromatograph the Standard solution, and record the peak responses as directed for Procedure in the Assay: the resolution,  $R$ , between salicylic acid and aspirin is not less than 2.0; and the relative standard deviation of the salicylic acid peak responses is not more than 4.0%.

**Procedure**—Proceed as directed for Procedure in the Assay. The relative retention times are about 0.7 for salicylic acid and 1.0 for aspirin. Calculate the percentage of salicylic acid ( $C_7H_6O_3$ ) in the portion of Tablets taken by the formula:

$$2000(C / Q_A)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Salicylic Acid RS in the Standard solution;  $Q_A$  is the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken, as determined in the Assay; and  $r_U$  and  $r_S$  are the peak responses of the salicylic acid peaks obtained from the Test solution and the Standard solution, respectively: not more than 3.0% is found.

#### Assay—

**Mobile phase**—Dissolve 2 g of sodium 1-heptanesulfonate in a mixture of 850 mL of water and 150 mL of acetonitrile, and adjust with glacial acetic acid to a pH of 3.4.

**Diluting solution**—Prepare a mixture of acetonitrile and formic acid (99:1).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Aspirin RS in Diluting solution to obtain a solution having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 100 mg of aspirin, to a suitable container. Add 20.0 mL of Diluting solution and about 10 glass beads. Shake vigorously for about 10 minutes, and centrifuge (Stock solution). Quantitatively dilute an accurately measured volume of the Stock solution with 9 volumes of Diluting solution (Assay preparation). Retain the remaining portion of Stock solution for the test for Limit of free salicylic acid.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm  $\times$  30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the Standard preparation, and record the peak responses as directed for Procedure: the tailing factor is not greater than 2.0; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the formula:

$$200C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Aspirin RS in the Standard preparation; and  $r_U$  and  $r_S$  are the peak responses of the aspirin peaks obtained from the Assay preparation and the Standard preparation, respectively.

## Aspirin, Alumina, and Magnesia Tablets

» Aspirin, Alumina, and Magnesia Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aspirin ( $C_9H_8O_4$ ), the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of aluminum hydroxide [ $Al(OH)_3$ ], and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of magnesium hydroxide [ $Mg(OH)_2$ ].

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Aspirin RS

USP Salicylic Acid RS

#### Identification—

**A:** The chromatogram of the Assay preparation obtained as directed in the Assay for aspirin and limit of free salicylic acid exhibits a major peak for aspirin, the retention time of which corresponds with that exhibited in the chromatogram of the Standard preparation, obtained as directed in the Assay for aspirin and limit of free salicylic acid.

**B:** To a 0.7-g portion of finely powdered Tablets add 20 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution changes to deep yellow. Continue boiling for 2 minutes, and filter: the filtrate so obtained responds to the tests for Magnesium (191).

**C:** Wash the precipitate obtained in Identification test B with a hot solution of ammonium chloride (1 in 50), and dissolve the precipitate in hydrochloric acid: the solution so obtained responds to the tests for Aluminum (191).

#### Dissolution (711)—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate (trihydrate) and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 900 mL.

**Apparatus 2:** 75 rpm.

**Time:** 45 minutes.

**Procedure**—Determine the amount of aspirin ( $C_9H_8O_4$ ) dissolved from UV absorbances at the wavelength of the isosbestic point of aspirin and salicylic acid at  $265 \pm 2$  nm of filtered portions of the solution under test, suitably diluted with Medium, if necessary, in comparison with a Standard



solution having a known concentration of USP Aspirin RS in the same medium. [NOTE—Prepare the Standard solution at the time of use. An amount of methanol not to exceed 1% of the total volume of the Standard solution may be used to bring the Reference Standard into solution prior to dilution with Medium.]

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_9H_8O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium hydroxide, and for *Content Uniformity* with respect to aspirin.

**Acid-neutralizing capacity** (301): not less than 1.9 mEq of acid is consumed for each 325 mg of aspirin in the Tablets.

#### Assay for aspirin and limit of free salicylic acid—

**Mobile phase**—Dissolve 225 mg of tetramethylammonium hydroxide pentahydrate and 200 mg of sodium 1-octanesulfonate in 700 mL of water. Add 150 mL of methanol, 150 mL of acetonitrile, and 1.0 mL of glacial acetic acid, and stir. [NOTE—The composition of the *Mobile phase* may be adjusted if necessary (see *System Suitability* under *Chromatography* (621)).]

**Solvent mixture**—To 2 g of anhydrous citric acid add 990 mL of acetonitrile, 990 mL of chloroform, and 20 mL of formic acid, and stir for about 30 minutes. Allow to settle, and decant the clear solution into a suitable container. Use the clear solution as the *Solvent mixture*.

**Internal standard solution**—Dissolve phenacetin in *Solvent mixture* to obtain a solution having a concentration of about 2 mg per mL.

**Salicylic acid stock standard solution**—Dissolve a suitable quantity of USP Salicylic Acid RS in *Solvent mixture* to obtain a solution having a known concentration of about 1 mg per mL.

**Standard preparation**—Transfer about 325 mg of USP Aspirin RS, accurately weighed, to a 50-mL volumetric flask. Add 10.0 mL of *Salicylic acid stock standard solution* and 5.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Immediately transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a screw-capped, 120-mL bottle, add 5.0 mL of *Internal standard solution* and 45.0 mL of *Solvent mixture*, cap the bottle, mix, and sonicate for 2 to 5 minutes. Centrifuge, and use a portion of the resultant clear solution as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm  $\times$  30-cm column that contains 10- $\mu$ m packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.3 for salicylic acid, 0.6 for aspirin, and 1.0 for phenacetin. [NOTE—Record each chromatogram until the chloroform peak appears at a relative retention time of about 1.8;] the resolution, *R*, between the salicylic acid, aspirin, and internal standard peaks is not less than 2.0; the tailing factor for any of these peaks is not more than 2.0; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

tity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the formula:

$$50C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Aspirin RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of aspirin and phenacetin obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentage of free salicylic acid in the Tablets taken by the formula:

$$5000(C / a)(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Standard preparation*; *a* is the quantity, in mg, of aspirin in the portion of Tablets taken, based on the labeled amount; and  $R_U$  and  $R_S$  are the ratios of the peak responses of salicylic acid and phenacetin obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 3.0% is found.

#### Assay for aluminum hydroxide—

**Eдетate disodium titrant**—Prepare and standardize as directed in the *Assay under Ammonium Alum*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 250 mg of aluminum hydroxide, to a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the flask, add water to volume, and mix.

**Procedure**—Pipet 50 mL of *Assay preparation* into a 250-mL beaker, then add, in the order named and with continuous stirring, 25.0 mL of 0.05 M *Eдетate disodium titrant* and 20 mL of acetic acid-ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 minutes. Cool, add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 50 mL of water for the *Assay preparation*, and make any necessary corrections. Each mL of 0.05 M *Eдетate disodium titrant* consumed is equivalent to 3.900 mg of  $Al(OH)_3$ .

#### Assay for magnesium hydroxide—

**Assay preparation**—Prepare as directed in the *Assay for aluminum hydroxide*.

**Procedure**—Pipet a volume of *Assay preparation*, equivalent to about 80 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and mix. Add 50 mL of ammonia-ammonium chloride buffer TS and 2 drops of eriochrome black indicator solution (prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mixing). Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M *edetate disodium VS* until the color changes to pure blue. Perform a blank determination, substituting for the *Assay preparation*, a volume of water equal to the volume of *Assay preparation* used, and make any necessary corrections. Each mL of 0.05 M *edetate disodium* is equivalent to 2.916 mg of  $Mg(OH)_2$ .

## Aspirin, Alumina, and Magnesium Oxide Tablets

### DEFINITION

Aspirin, Alumina, and Magnesium Oxide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of aspirin ( $C_9H_8O_4$ ), the equivalent of NLT 90.0% and NMT



110.0% of the labeled amount of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , and NLT 90.0% and NMT 110.0% of the labeled amount of magnesium oxide ( $\text{MgO}$ ).

## IDENTIFICATION

**Sample:** The *Sample* is prepared as follows. To a 0.7-g portion of finely powdered Tablets, add 20 mL of 3 N hydrochloric acid and 5 drops of methyl red TS, heat to boiling, and add 6 N ammonium hydroxide until the color of the solution changes to deep yellow. Continue boiling for 2 min, and filter. The filtrate is used in *Identification* test B, and the precipitate is used in *Identification* test C.

- **A.** The retention time of the aspirin peak of the *Sample* solution corresponds to that of the *Standard* solution, as obtained in the *Assay*.

- **B. IDENTIFICATION TESTS—GENERAL, Magnesium (191)**

**Sample solution:** *Sample* filtrate

**Acceptance criteria:** Meet the requirements

- **C. IDENTIFICATION TESTS—GENERAL, Aluminum (191)**

**Sample solution:** Wash the *Sample* precipitate with a hot solution of 20 mg/mL of ammonium chloride, and dissolve the precipitate in hydrochloric acid.

**Acceptance criteria:** Meet the requirements

- **D. PROCEDURE**

**Analysis:** Where the Tablets are composed of two layers, scrape a small amount of each layer into separate test tubes. Add 2 mL of water and 2 drops of methyl red TS to each tube, and shake for 15 s.

**Acceptance criteria:** The solution from the aspirin-containing layer is red, and the solution from the buffer-containing layer is yellow.

## ASSAY

- **ASPIRIN**

**Mobile phase:** Methanol, phosphoric acid, and water (30:3:70)

**Diluent:** Dehydrated alcohol and hydrochloric acid (2000:20)

**Aspirin standard stock solution:** 5 mg/mL of USP Aspirin RS in *Diluent* prepared by blending at high speed for 1.5 min

**Aspirin standard solution:** 0.25 mg/mL of USP Aspirin RS prepared immediately from the *Aspirin standard stock solution* in dehydrated alcohol. Use these solutions within 1 h.

**Salicylic acid standard stock solution:** 5 mg/mL of USP Salicylic Acid RS in dehydrated alcohol. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

**Salicylic acid standard solution:** 7.5  $\mu\text{g}$ /mL of USP Salicylic Acid RS from the *Salicylic acid standard stock solution* in dehydrated alcohol

**System suitability solution:** Transfer 5.0 mL of the *Aspirin standard stock solution* to a 100-mL volumetric flask, add 5.0 mL of the *Salicylic acid standard stock solution*, and dilute with dehydrated alcohol to volume.

**Sample solution:** Transfer a counted number of Tablets, equivalent to 2500 mg of aspirin, to a 120-mL blender jar containing 100.0 mL of *Diluent*, and blend at high speed for 1.5 min. Immediately filter a portion of the mixture thus obtained, and transfer 1.0 mL of the filtrate to a 100-mL volumetric flask. Immediately dilute with dehydrated alcohol to volume. Promptly inject this *Sample solution* into the chromatograph as directed for *Analysis*.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 205 nm

**Column:** 4.6-mm  $\times$  3-cm; 5- $\mu\text{m}$  packing L7

**Flow rate:** 3.5 mL/min

**Injection volume:** 10  $\mu\text{L}$

### System suitability

**Samples:** *Aspirin standard solution*, *Salicylic acid standard solution*, and *System suitability solution*

[NOTE—The relative retention times for aspirin and salicylic acid are 0.7 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 2.0 between the aspirin peak and the salicylic acid peak, *System suitability solution*

**Tailing factor:** NMT 2.0 for the aspirin and salicylic acid peaks, *Aspirin standard solution* and *Salicylic acid standard solution*

**Relative standard deviation:** NMT 2.0% for the aspirin and salicylic acid peaks, *Aspirin standard solution* and *Salicylic acid standard solution*

### Analysis

**Samples:** *Aspirin standard solution*, *Salicylic acid standard solution*, and *Sample solution*

Calculate the percentage of aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ) in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of aspirin from the *Sample solution*

$r_S$  = peak response of aspirin from the *Standard solution*

$C_S$  = concentration of USP Aspirin RS in the *Aspirin standard solution* (mg/mL)

$C_U$  = nominal concentration of aspirin in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

- **ALUMINUM HYDROXIDE**

**Edetate disodium titrant:** Dissolve 18.6 g of edetate disodium in water to make 1000 mL, and standardize the solution as follows. Weigh 2 g of aluminum wire, transfer to a 1000-mL volumetric flask, and add 50 mL of a mixture of hydrochloric acid and water (1:1). Swirl the flask to ensure contact of the aluminum and the acid, and allow the reaction to proceed until all of the aluminum has dissolved. Dilute with water to volume. Pipet 10 mL of this solution into a 250-mL beaker; add, in the order named and with continuous stirring, 25.0 mL of edetate disodium titrant and 20 mL of acetic acid–ammonium acetate buffer TS; and boil gently for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate with 0.05 M zinc sulfate VS to a bright rose-pink color. Perform a blank determination, substituting 10 mL of water for the aluminum solution, and make any necessary correction.

Calculate the molarity of the solution taken:

$$\text{Result} = (W/A_r) \times V$$

$W$  = weight of aluminum in the portion of the solution taken (mg)

$A_r$  = atomic weight of aluminum, 26.98

$V$  = volume of *Edetate disodium titrant* consumed (mL)

**Sample solution:** To a portion of the powdered Tablets (NLT 20) equivalent to 600 mg of aluminum hydroxide, add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and transfer to a 200-mL volumetric flask. Wash the beaker with water, adding the washings to the flask, and add water to volume.

**Analysis:** To 20 mL of the *Sample solution* in a 250-mL beaker, add 20 mL of water; then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid–ammonium acetate buffer TS, and heat the solution near the boiling temperature for 5 min. Cool, and add 50 mL of alcohol and 2 mL of dithizone TS. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Sample solution*, and make any necessary correction. Each mL of 0.05 M *Edetate disodium titrant* is equivalent to 3.900 mg of aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ .



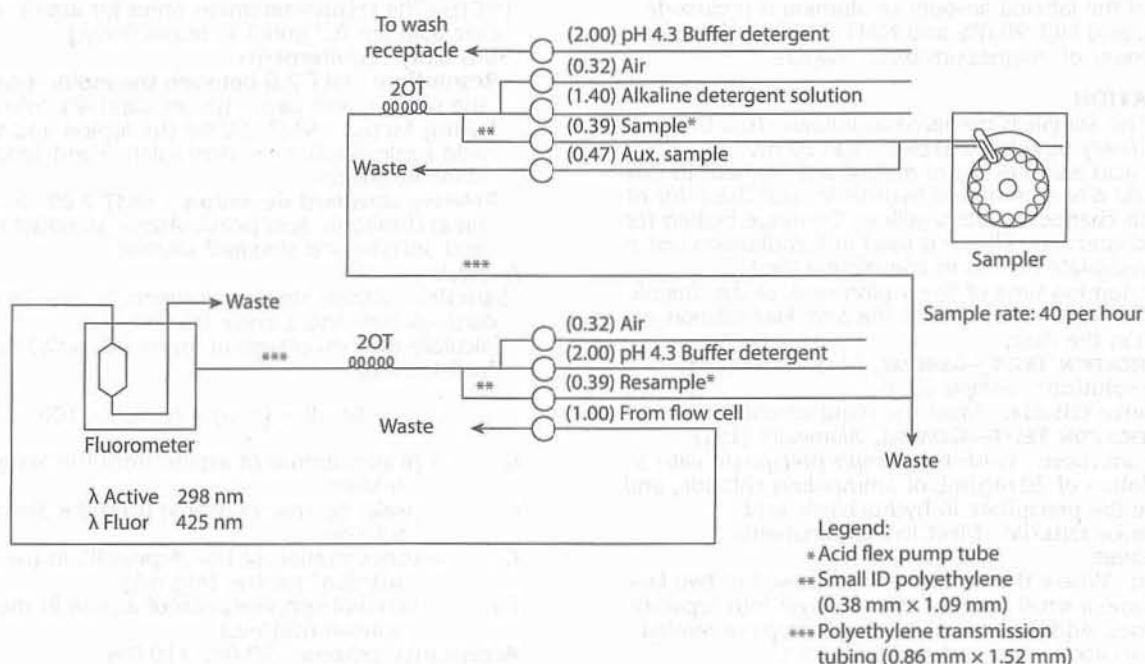


Figure 1

Acceptance criteria: 90.0%–110.0%

#### • MAGNESIUM OXIDE

**Sample solution:** Prepare as directed in the Assay for Aluminum Hydroxide.

**Eriochrome black indicator solution:** Dissolve 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol.

**Titrant:** 0.05 M edetate disodium VS

**Analysis:** To a volume of the *Sample solution* equivalent to 40 mg of magnesium oxide in a 400 mL beaker, add, while mixing, 20 mL of triethanolamine and 200 mL of water. Cool the solution for 10 min, while stirring, by immersion in an ice bath. Remove the beaker from the ice bath, and add 15 mL of ammonia–ammonium chloride buffer TS and 2 drops of *Eriochrome black indicator solution*. Titrate with *Titrant* to a blue endpoint, allowing 60 s between drops of *Titrant* as the endpoint is approached (after first color change is observed). The titration should be completed within 10 min after the addition of the buffer and indicator. If any precipitate is observed prior to titration, the solution should be discarded, and a new solution prepared. Perform a blank determination, substituting an equivalent volume of water for the volume of *Sample solution* used, and make any necessary correction. Each mL of *Titrant* consumed is equivalent to 2.015 mg of magnesium oxide (MgO).

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate (trihydrate) and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 900 mL

**Apparatus 1 (10-mesh screen):** 100 rpm

**Time:** 45 min

Determine the amount of aspirin ( $C_9H_8O_4$ ) dissolved, employing the following method.

**Alkaline detergent solution:** 30% solution of polyoxyethylene (23) lauryl ether and 1 N sodium hydroxide (0.5:1000)

**pH 4.3 buffer detergent:** 12.9 g/L of citric acid monohydrate and 20.6 g/L of dibasic sodium phosphate

heptahydrate in water. Add 0.5 mL of a 30% solution of polyoxyethylene (23) lauryl ether.

**Standard solution:** 0.45 mg/mL of USP Aspirin RS in Medium

**Sample solution:** Filtered portion of sample

**Analysis:** Use an automatic analyzer consisting of (1) a liquid sampler, (2) a proportioning pump, (3) a suitable fluorometer equipped with a 0.4-cm flow cell and suitable recording devices, and (4) a manifold consisting of the components illustrated in Figure 1.

With the sample line pumping pH 4.3 buffer detergent, the other lines pumping their respective reagents, and the fluorometer set at an excitation wavelength of 298 nm and an emission wavelength of 425 nm, adjust the system until a steady fluorescence baseline has been achieved. Start the sampler, and conduct determinations at a rate of 40/h, using a ratio of 5:1 for sample and wash time. Record the fluorescence values of the *Standard solution* and the *Sample solution*.

Calculate the percentage of aspirin ( $C_9H_8O_4$ ) dissolved:

$$\text{Result} = C_s \times (V/L) \times (F_u/F_s) \times 100$$

$C_s$  = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)

$V$  = volume of medium, 900 mL

$L$  = label claim (mg/Tablet)

$F_u$  = fluorescence values of the solution under test

$F_s$  = fluorescence values of the *Standard solution*

**Tolerances:** NLT 75% (Q) of the labeled amount of aspirin ( $C_9H_8O_4$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation* with respect to aluminum hydroxide and to magnesium oxide, and for *Content Uniformity* with respect to aspirin

#### IMPURITIES

##### • ORGANIC IMPURITIES PROCEDURE: LIMIT OF FREE SALICYLIC ACID

[NOTE—The results from the Assay for Aspirin may be used for this test when calculated as described in the Analysis section of this test.]



**Mobile phase:** Methanol, phosphoric acid, and water (30:3:70)

**Diluent:** Dehydrated alcohol and hydrochloric acid (2000:20)

**Aspirin standard stock solution:** 5 mg/mL of USP Aspirin RS in *Diluent* prepared by blending at high speed for 1.5 min

**Aspirin standard solution:** 0.25 mg/mL of USP Aspirin RS prepared immediately from *Aspirin standard stock solution* in dehydrated alcohol. Use these solutions within 1 h.

**Salicylic acid standard stock solution:** 5 mg/mL of USP Salicylic Acid RS in dehydrated alcohol. Transfer 3.0 mL of this solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume.

**Salicylic acid standard solution:** 7.5 µg/mL of USP Salicylic Acid RS from *Salicylic acid standard stock solution* in dehydrated alcohol

**System suitability solution:** Transfer 5.0 mL of the *Aspirin standard stock solution* to a 100-mL volumetric flask, add 5.0 mL of the *Salicylic acid standard stock solution*, and dilute with dehydrated alcohol to volume.

**Sample solution:** Transfer a counted number of Tablets, equivalent to 2500 mg of aspirin, to a 120-mL blender jar containing 100.0 mL of *Diluent*, and blend at high speed for 1.5 min. Immediately filter a portion of the mixture thus obtained, and transfer 1.0 mL of the filtrate to a 100-mL volumetric flask. Immediately dilute with dehydrated alcohol to volume. Promptly inject this *Sample solution* into the chromatograph as directed for *Analysis*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 205 nm

**Column:** 4.6-mm × 3-cm; 5-µm packing L7

**Flow rate:** 3.5 mL/min

**Injection volume:** 10 µL

#### System suitability

**Samples:** *Aspirin standard solution*, *Salicylic acid standard solution*, and *System suitability solution*

[NOTE—The relative retention times for aspirin and salicylic acid are 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between the aspirin and salicylic acid peaks, *System suitability solution*

**Tailing factor:** NMT 2.0 for the aspirin and salicylic acid peaks, *Aspirin standard solution* and *Salicylic acid standard solution*

**Relative standard deviation:** NMT 2.0% for aspirin and salicylic acid peaks, *Aspirin standard solution* and *Salicylic acid standard solution*

#### Analysis

**Samples:** *Aspirin standard solution*, *Salicylic acid standard solution*, and *Sample solution*

Calculate the percentage of free salicylic acid in the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of salicylic acid from the *Sample solution*

$r_S$  = peak response from the *Salicylic acid standard solution*

$C_S$  = concentration of USP Salicylic Acid RS in the *Salicylic acid standard solution* (µg/mL)

$C_U$  = nominal concentration of aspirin in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 3.0%

#### SPECIFIC TESTS

- **ACID-NEUTRALIZING CAPACITY (301):** NLT 1.9 mEq of acid is consumed for each 325 mg of aspirin in the Tablets.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
  - USP Aspirin RS
  - USP Salicylic Acid RS

### Aspirin, Caffeine, and Dihydrocodeine Bitartrate Capsules

» Aspirin, Caffeine, and Dihydrocodeine Bitartrate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aspirin ( $C_9H_8O_4$ ), caffeine ( $C_8H_{10}N_4O_2$ ), and dihydrocodeine bitartrate ( $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$ ).

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Aspirin RS

USP Caffeine RS

USP Dihydrocodeine Bitartrate RS

USP Salicylic Acid RS

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

#### Dissolution, Procedure for a Pooled Sample (711)—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 500 mL.

**Apparatus 1:** 50 rpm.

**Time:** 45 minutes.

**Mobile phase and Chromatographic system**—Prepare as directed in the *Assay and limit of salicylic acid*.

**Standard preparation**—Prepare a solution in *Medium* containing known concentrations of about 0.002A mg of USP Aspirin RS, 0.002C mg of USP Caffeine RS, and 0.002D mg of USP Dihydrocodeine Bitartrate RS per mL, A, C, and D being the labeled amounts, in mg, of aspirin, caffeine, and dihydrocodeine bitartrate, respectively, in each Capsule.

**Test preparation**—Filter a portion of the solution under test.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of aspirin ( $C_9H_8O_4$ ), caffeine ( $C_8H_{10}N_4O_2$ ), and dihydrocodeine bitartrate ( $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$ ) dissolved by the same formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the relevant analyte obtained from the *Test preparation* and the *Standard preparation*, respectively.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of  $C_9H_8O_4$ ,  $C_8H_{10}N_4O_2$ , and  $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$  are dissolved in 45 minutes.

**Uniformity of dosage units (905):** meet the requirements.

#### Assay and limit of salicylic acid—

**Mobile phase**—Dissolve 1 g of sodium 1-pentanesulfonate and 2.3 g of monobasic ammonium phosphate in 850 mL of water. Add 150 mL of acetonitrile, mix, degas, and adjust



with phosphoric acid to a pH of 2.5. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of water and acetonitrile (53:46), and adjust with phosphoric acid to a pH of 2.5.

**Standard preparation**—Prepare a solution in *Diluent* containing known concentrations of about 0.001A mg of USP Aspirin RS, 0.001C mg of USP Caffeine RS, and 0.001D mg of USP Dihydrocodeine Bitartrate RS per mL, A, C, and D being the labeled amounts, in mg, of aspirin, caffeine, and dihydrocodeine bitartrate, respectively, in each Capsule. [NOTE—Use this solution within 3 hours.]

**Standard salicylic acid preparation**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in *Diluent* to obtain a solution having a known concentration of about 0.005A µg per mL, A being the labeled amount, in mg, of aspirin per Capsule. [NOTE—Use this solution within 3 hours.]

**Resolution solution**—Prepare a solution in *Standard preparation* containing about 0.0001A mg of USP Salicylic Acid RS per mL, A being the labeled amount, in mg, of aspirin in each Capsule. [NOTE—Use this solution within 3 hours.]

**Assay preparation**—Transfer the contents of 10 Capsules to a 500-mL volumetric flask. Dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this mixture to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Centrifuge a portion of this mixture, and use the clear supernatant as the *Assay preparation*. [NOTE—Use this solution within 3 hours.]

**Chromatographic system**—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times are about 0.2 for caffeine, 0.3 for dihydrocodeine, 0.7 for aspirin, and 1.0 for salicylic acid; and the resolution, *R*, between the caffeine and dihydrocodeine peaks is not less than 2.5, between the dihydrocodeine and aspirin peaks is not less than 1.0, and between the aspirin and salicylic acid peaks is not less than 1.5. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0% for each analyte.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Assay preparation*, the *Standard preparation*, and the *Standard salicylic acid preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of aspirin ( $C_9H_8O_4$ ), caffeine ( $C_8H_{10}N_4O_2$ ), and dihydrocodeine bitartrate ( $C_{18}H_{23}NO_3 \cdot C_4H_6O_6$ ) in each Capsule taken by the same formula:

$$1000C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_u$  and  $r_s$  are the responses of the corresponding analyte peaks of the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentage of salicylic acid in the Capsules taken by the formula:

$$100(C / A)(r_u / r_s)$$

in which C is the concentration, in µg per mL, of USP Salicylic Acid RS in the *Standard salicylic acid preparation*; A is the labeled amount, in mg, of aspirin in each Capsule taken; and  $r_u$  and  $r_s$  are the salicylic acid peak responses obtained from the *Assay preparation* and the *Standard salicylic acid preparation*, respectively: not more than 3.0% is found.

## Aspirin and Codeine Phosphate Tablets

» Aspirin and Codeine Phosphate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aspirin ( $C_9H_8O_4$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

### USP Reference standards (11)—

USP Aspirin RS

USP Codeine Phosphate RS

USP Salicylic Acid RS

**Identification**—Dissolve a suitable quantity of USP Aspirin RS in the *Solvent mixture* prepared as directed under *Assay for aspirin and codeine phosphate and limit of free salicylic acid* to obtain a *Standard aspirin solution* containing about 3.3 mg per mL. Dissolve a suitable quantity of USP Codeine Phosphate RS in the *Solvent mixture* to obtain a *Standard codeine phosphate solution* containing about 1 mg per mL. Chromatograph these solutions as directed for *Procedure* in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid*. The retention times of the major peaks in the chromatogram of the *Assay preparation*, obtained as directed in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid*, correspond to those in the chromatograms of the *Standard aspirin solution* and the *Standard codeine phosphate solution*, respectively.

### Dissolution (711)—

**Medium**: 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 900 mL.

**Apparatus 2**: 75 rpm.

**Time**: 30 minutes.

Determine the amounts of aspirin ( $C_9H_8O_4$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) dissolved by employing the following method.

**Mobile phase, Solvent mixture, and Aspirin and codeine phosphate standard preparation**—Prepare as directed in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid*.

**Internal standard solution**—Dissolve phenacetin in methanol to obtain a solution having a concentration of about 0.07 mg per mL.

**Standard solution A**—Prepare a solution of USP Aspirin RS in *Solvent mixture* having an accurately known concentration of about 0.36 mg per mL.

**Standard solution B**—Transfer about 12 mg of USP Codeine Phosphate RS and 25 mg of USP Salicylic Acid RS, each accurately weighed, to a 50-mL volumetric flask, add 2.5 mL of methanol, and mix. Add *Medium* to volume, and mix. Pipet 10 mL of the resulting solution into a 100-mL volumetric flask, add *Medium* to volume, and mix.

**Standard preparations A and B**—Pipet 10 mL of *Standard solution A* and 10 mL of *Standard solution B* into separate containers, add 3.0 mL of the *Internal standard solution* to each container, and mix.

**Test preparation**—Withdraw a portion of the solution under test and filter, discarding the few mL of the filtrate. Pipet 10 mL of the filtrate and 3.0 mL of the *Internal standard solution* into a suitable container, and mix.

**Chromatographic system**—Proceed as directed for *Chromatographic system* in the *Assay for aspirin and codeine phosphate and limit of free salicylic acid*, except to use only the *Aspirin and codeine phosphate preparation* for evaluation of the suitability of the system.



**Procedure**—Proceed as directed in the Assay for aspirin and codeine phosphate and limit of free salicylic acid, except to inject about 50  $\mu$ L of the Standard preparations and the Test preparation. The relative retention times are 0.3 for salicylic acid, 0.6 for aspirin, 0.8 for codeine phosphate, and 1.0 for phenacetin. Calculate the amount of codeine phosphate dissolved by comparison of the relative peak response ratios for the codeine phosphate peaks, obtained from Standard preparation B and the Test preparation. Calculate the percentage of aspirin dissolved by the formula:

$$[0.9C(R_U / R_S) + 0.9C'(R'_U / R'_S)(180.16 / 138.12)] / 3.25$$

in which C is the concentration, in  $\mu$ g per mL, of USP Aspirin RS in Standard solution A;  $R_U$  and  $R_S$  are the peak response ratios for the aspirin component obtained from the Test preparation and Standard preparation A, respectively;  $C'$  is the concentration, in  $\mu$ g per mL, of USP Salicylic Acid RS in Standard solution B;  $R'_U$  and  $R'_S$  are the peak response ratios for the salicylic acid component obtained from the Test preparation and Standard preparation B, respectively; and 180.16 and 138.12 are the molecular weights of aspirin and salicylic acid, respectively.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of aspirin ( $C_9H_8O_4$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements for Content Uniformity with respect to aspirin and codeine phosphate.

**Assay for aspirin and codeine phosphate and limit of free salicylic acid**—

**Mobile phase**—Dissolve 225 mg of tetramethylammonium hydroxide pentahydrate and 200 mg of sodium 1-octanesulfonate in 700 mL of water. Add 150 mL of methanol, 150 mL of acetonitrile, and 1.0 mL of glacial acetic acid, and stir. Pass through a membrane filter, and degas. [NOTE—The amounts of sodium 1-octanesulfonate, methanol, and acetonitrile may be varied to obtain acceptable chromatography.]

**Solvent mixture**—To 15 g of anhydrous citric acid add 200 mL of methanol and 20 mL of glacial acetic acid, dilute with chloroform to 1000 mL, and mix until the citric acid is dissolved.

**Internal standard solution**—Dissolve phenacetin in Solvent mixture to obtain a solution having a concentration of about 2 mg per mL.

**Salicylic acid stock standard solution**—Dissolve an accurately weighed quantity of USP Salicylic Acid RS in Solvent mixture, and quantitatively dilute with Solvent mixture to obtain a solution having a known concentration of about 1 mg per mL.

**Salicylic acid standard preparation**—Transfer 5.0 mL of Salicylic acid stock standard solution to a 50-mL volumetric flask, add 5.0 mL of Internal standard solution, dilute with Solvent mixture to volume, and mix.

**Codeine phosphate stock standard solution**—Transfer about 325J mg of USP Codeine Phosphate RS, accurately weighed, to a 25-mL volumetric flask, J being the ratio of the labeled amount, in mg, of codeine phosphate to the labeled amount, in mg, of aspirin per Tablet. Dissolve in and dilute with Solvent mixture to volume, and mix.

**Aspirin and codeine phosphate standard preparation**—Transfer about 65 mg of USP Aspirin RS, accurately weighed, to a 10-mL volumetric flask. Add 5.0 mL of Codeine phosphate stock standard solution, 1.0 mL of Salicylic acid stock standard solution, and 1.0 mL of Internal standard solution, dilute with Solvent mixture to volume, and mix.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a screw-capped, 120-mL bottle, add 5.0 mL of Internal standard solution and 45.0 mL of Solvent mixture, mix, and soni-

cate for 2 to 5 minutes. Centrifuge, and use a portion of the resultant clear solution as the Assay preparation. Use on the day prepared.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm  $\times$  30-cm column that contains 10- $\mu$ m packing L1. The flow rate is about 2 mL per minute. Chromatograph replicate injections of the Salicylic acid standard preparation and the Aspirin and codeine phosphate standard preparation, and record the peak responses as directed for Procedure: the relative retention times for salicylic acid, aspirin, codeine, and phenacetin are about 0.3, 0.5, 0.8, and 1.0, respectively; the resolution,  $R$ , between salicylic acid and aspirin, between aspirin and codeine, and between codeine and phenacetin is not less than 2.0; the tailing factor for each analyte peak is not more than 2.0; and the relative standard deviation of the ratios of the peak responses of salicylic acid, aspirin, and codeine to the peak response of phenacetin is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the Salicylic acid standard preparation, Aspirin and codeine phosphate standard preparation, and Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Aspirin RS in the Aspirin and codeine phosphate standard preparation; and  $R_U$  and  $R_S$  are the ratios of the peak responses of aspirin and phenacetin obtained from the Assay preparation and the Aspirin and codeine phosphate standard preparation, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Tablets taken by the formula:

$$(406.37/397.37)(50C)(R_U / R_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; C is the concentration, in mg per mL, of USP Codeine Phosphate RS in the Aspirin and codeine phosphate standard preparation; and  $R_U$  and  $R_S$  are the ratios of the peak responses of codeine phosphate and phenacetin obtained from the Assay preparation and the Aspirin and codeine phosphate standard preparation, respectively. Calculate the percentage of free salicylic acid in the Tablets taken by the formula:

$$5000(C/a)(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the Salicylic acid standard preparation; a is the quantity, in mg, of aspirin in the portion of powdered Tablets taken, based on the labeled amount; and  $R_U$  and  $R_S$  are the ratios of the peak responses of salicylic acid and phenacetin obtained from the Assay preparation and the Salicylic acid standard preparation, respectively: not more than 3.0% is found.

## Aspirin, Codeine Phosphate, Alumina, and Magnesia Tablets

» Aspirin, Codeine Phosphate, Alumina, and Magnesia Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of aspirin ( $C_9H_8O_4$ ), codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ),



aluminum hydroxide  $[\text{Al}(\text{OH})_3]$ , and magnesium hydroxide  $[\text{Mg}(\text{OH})_2]$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Aspirin RS  
USP Codeine Phosphate RS  
USP Salicylic Acid RS

**Identification**—

**A:** Tablets respond to the Identification test under *Aspirin and Codeine Phosphate Tablets*.

**B:** Tablets respond to the Identification tests under *Alumina and Magnesia Tablets*.

**Dissolution** (711)—

**Medium:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate trihydrate and 1.66 mL of glacial acetic acid with water to obtain 1000 mL of solution having a pH of  $4.50 \pm 0.05$ ; 900 mL.

**Apparatus 2:** 75 rpm.

**Time:** 30 minutes.

**Mobile phase, Internal standard solution, Solvent mixture, Aspirin and codeine phosphate standard preparation, Standard solution A, Standard solution B, Standard preparations A and B, Test preparation, Chromatographic system, and Procedure**—Proceed as directed in the test for Dissolution under *Aspirin and Codeine Phosphate Tablets*.

**Tolerances**—Not less than 75% (Q) of the labeled amounts of aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ) and codeine phosphate hemihydrate ( $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ) are dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity* with respect to aspirin and codeine phosphate and for *Weight Variation* with respect to aluminum hydroxide and magnesium hydroxide.

**Acid-neutralizing capacity** (301): not less than 1.9 mEq per Tablet.

**Assay for aspirin and codeine phosphate and limit of free salicylic acid**—

**Mobile phase, Solvent mixture, Salicylic acid stock standard solution, Salicylic acid standard preparation, Aspirin and codeine phosphate standard preparation, and Chromatographic system**—Prepare as directed in the Assay for aspirin and codeine phosphate and limit of free salicylic acid under *Aspirin and Codeine Phosphate Tablets*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 325 mg of aspirin, to a screw-capped, 120-mL bottle, add 5.0 mL of *Internal standard solution* and 45.0 mL of *Solvent mixture*, mix, and sonicate for 2 to 5 minutes. Centrifuge, and use a portion of the resultant clear solution as the *Assay preparation*.

**Procedure**—Separately inject equal volumes (about 5  $\mu\text{L}$ ) of the *Salicylic acid standard preparation*, the *Aspirin and codeine phosphate standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times for salicylic acid, aspirin, codeine, and phenacetin are about 0.3, 0.5, 0.8, and 1.0, respectively. Calculate the quantity, in mg, of aspirin ( $\text{C}_9\text{H}_8\text{O}_4$ ) in the portion of powdered Tablets taken by the formula:

$$50C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Aspirin RS in the *Aspirin and codeine phosphate standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of

aspirin and phenacetin obtained from the *Assay preparation* and the *Aspirin and codeine phosphate standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ ), in the portion of powdered Tablets taken by the formula:

$$(406.37/397.37)(50C)(R_U / R_S)$$

in which 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; C is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Aspirin and codeine phosphate standard preparation*; and  $R_U$  and  $R_S$  are the ratios of the peak responses of codeine phosphate and phenacetin obtained from the *Assay preparation* and the *Aspirin and codeine phosphate Standard preparation*, respectively. Calculate the percentage of free salicylic acid in the Tablets taken by the formula:

$$5000(C/a)(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Salicylic Acid RS in the *Salicylic acid standard preparation*; a is the quantity, in mg, of aspirin in the portion of Tablets taken, determined as directed above; and  $R_U$  and  $R_S$  are the ratios of the peak responses of salicylic acid and phenacetin obtained from the *Assay preparation* and the *Salicylic acid standard preparation*, respectively: not more than 3.0% is found.

**Assay for aluminum hydroxide**—

**Edetate disodium titrant**—Prepare and standardize as directed in the Assay under *Ammonium Alum*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600 mg of aluminum hydroxide, to a 150-mL beaker, add 20 mL of water, stir, and slowly add 30 mL of 3 N hydrochloric acid. Heat gently, if necessary, to aid solution, cool, and filter into a 200-mL volumetric flask. Wash the filter with water into the flask, add water to volume, and mix.

**Procedure**—Pipet 10 mL of *Assay preparation* into a 250-mL beaker, add 20 mL of water, then add, in the order named and with continuous stirring, 25.0 mL of *Edetate disodium titrant* and 20 mL of acetic acid-ammonium acetate buffer TS. Add 50 mL of alcohol and 2 mL of dithizone TS, and mix. Titrate with 0.05 M zinc sulfate VS until the color changes from green-violet to rose-pink. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M Edetate disodium titrant is equivalent to 3.900 mg of  $\text{Al}(\text{OH})_3$ .

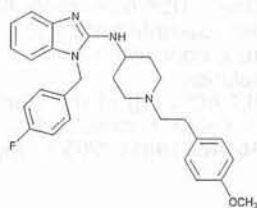
**Assay for magnesium hydroxide**—

**Assay preparation**—Prepare as directed in the Assay for aluminum oxide.

**Procedure**—Pipet a volume of *Assay preparation*, equivalent to about 40 mg of magnesium hydroxide, into a 400-mL beaker, add 200 mL of water and 20 mL of triethanolamine, and stir. Add 10 mL of ammonia-ammonium chloride buffer TS and 3 drops of an eriochrome black indicator solution prepared by dissolving 200 mg of eriochrome black T in a mixture of 15 mL of triethanolamine and 5 mL of dehydrated alcohol, and mix. Cool the solution to between 3° and 4° by immersion of the beaker in an ice bath, then remove, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Perform a blank determination, substituting 10 mL of water for the *Assay preparation*, and make any necessary correction. Each mL of 0.05 M edetate disodium consumed is equivalent to 2.916 mg of  $\text{Mg}(\text{OH})_2$ .



## Astemizole



$C_{28}H_{31}FN_4O$  458.57

1*H*-Benzimidazol-2-amine, 1-[(4-fluorophenyl)methyl]-*N*-[1-[2-(4-methoxyphenyl)ethyl]-4-piperidinyl]-; 1-(*p*-Fluorobenzyl)-2-[[1-(*p*-methoxyphenethyl)-4-piperidinyl]amino]benzimidazole [68844-77-9].

### DEFINITION

Astemizole contains NLT 98.0% and NMT 102.0% of astemizole ( $C_{28}H_{31}FN_4O$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, methanol, diethylamine, and 0.13 M ammonium acetate (230:470:1.0:300). Adjust with glacial acetic acid to a pH of 7.5.

**Standard solution:** 1.0 mg/mL of USP Astemizole RS in *Mobile phase*

**Sample solution:** 1.0 mg/mL of Astemizole in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 4000 theoretical plates

**Tailing factor:** NMT 1.8

**Relative standard deviation:** NMT 1.5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of astemizole ( $C_{28}H_{31}FN_4O$ ) in the portion of Astemizole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Astemizole RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Astemizole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

### Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 10 ppm • (Official 1, Jan-2018)

- **ORGANIC IMPURITIES**

**Solution A:** 17 g/L of tetrabutylammonium hydrogen sulfate in water

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	95	5
15	80	20
18	80	20
18.1	0	100
23	0	100

[NOTE—Equilibrate the system for 5 min before every injection.]

**System suitability solution:** 25 µg/mL of USP Astemizole RS and 250 µg/mL of ketoconazole in methanol

**Standard solution:** 25 µg/mL of USP Astemizole RS in methanol

**Sample solution:** 10 mg/mL of Astemizole in methanol

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 278 nm

**Column:** 4.6-mm × 10-cm; contains base-deactivated 3-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between astemizole and ketoconazole

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Astemizole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response of astemizole from the *Standard solution*

$C_S$  = concentration of USP Astemizole RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Astemizole in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Individual impurities:** NMT 0.25%

**Total impurities:** NMT 0.5%

### SPECIFIC TESTS

- **LOSS ON DRYING** (731)

**Analysis:** Dry under vacuum at 105° for 4 h.

**Acceptance criteria:** NMT 0.5%

- **MELTING RANGE OR TEMPERATURE** (741): 175°–178°

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS** (11)

USP Astemizole RS

## Astemizole Tablets

### DEFINITION

Astemizole Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of astemizole ( $C_{28}H_{31}FN_4O$ ).



**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHY**

**Standard solution:** 1 mg/mL of USP Astemizole RS in methanol

**Sample solution:** 1 mg/mL of Astemizole in methanol, prepared as follows. Transfer an amount of finely ground Tablets equivalent to 100 mg of Astemizole to a 100-mL volumetric flask, dilute with methanol to volume, and filter.

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Toluene, dioxane, methanol, and ammonium hydroxide (60:30:10:1)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Remove the plate from the developing chamber when the solvent front has moved about three-fourths of the length of the plate, mark the solvent front, air dry, and examine under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Acetonitrile, methanol, diethylamine, and 0.13 M ammonium acetate (230:470:1.0:300). Adjust with glacial acetic acid to a pH of 7.5.

**Standard solution:** 1 mg/mL of USP Astemizole RS in *Mobile phase*

**Sample solution:** Nominally 1 mg/mL of astemizole in *Mobile phase*, prepared as follows. Transfer an equivalent of 50 mg of astemizole from powdered Tablets (NLT 20) to a 50-mL volumetric flask. Add 25 mL of *Mobile phase*, mix for 30 min, dilute with *Mobile phase* to volume, and centrifuge. Use the supernatant.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 4000 theoretical plates

**Tailing factor:** NMT 1.8

**Relative standard deviation:** NMT 1.5%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of astemizole ( $C_{28}H_{31}FN_4O$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of astemizole from the *Sample solution*

$r_S$  = peak response of astemizole from the *Standard solution*

$C_S$  = concentration of USP Astemizole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of astemizole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION (711)**

**Medium:** Simulated gastric fluid TS (without the enzyme); 800 mL

**Apparatus 2:** 100 rpm

**Time:** 45 min

**Detector:** UV maximum at about 285 nm

**Standard solution:** USP Astemizole RS in *Medium*

**Sample solution:** Sample per *Dissolution* (711). Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

**Tolerances:** NLT 80% (Q) of the labeled amount of astemizole ( $C_{28}H_{31}FN_4O$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES**• **ORGANIC IMPURITIES**

**Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses of all of the peaks

**Acceptance criteria**

**Individual impurities:** NMT 0.25%

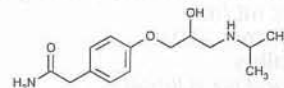
**Total impurities:** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Astemizole RS

**Atenolol**

$C_{14}H_{22}N_2O_3$

266.34

Benzeneacetamide, 4-[2-hydroxy-3-[(1-methylethyl)-amino]propoxy]-;

2-[p-[2-Hydroxy-3-(isopropylamino)propoxy]-phenyl]-acetamide [29122-68-7].

**DEFINITION**

Atenolol contains NLT 98.0% and NMT 102.0% of  $C_{14}H_{22}N_2O_3$ , calculated on the dried basis.

**IDENTIFICATION**

• **A. INFRARED ABSORPTION (197K)**

• **B. ULTRAVIOLET ABSORPTION (197U)**

**Sample solution:** 20  $\mu$ g/mL in methanol

**ASSAY**• **PROCEDURE**

**Mobile phase:** 1.1 g of sodium 1-heptanesulfonate and 0.71 g of anhydrous dibasic sodium phosphate in 700 mL of water. Add 2 mL of dibutylamine, and adjust with 0.8 M phosphoric acid to a pH of 3.0. Add 300 mL of methanol, mix, and pass through a filter having a 0.5- $\mu$ m or finer porosity. Degas this solution before use.

**Standard solution:** 0.01 mg/mL of USP Atenolol RS in *Mobile phase*

**Sample solution:** 0.01 mg/mL of Atenolol in *Mobile phase*. Sonicate for 5 min for complete dissolution.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 226 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 0.6 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 5000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{14}H_{22}N_2O_3$  in the portion of Atenolol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Atenolol RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Atenolol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

## IMPURITIES

### Inorganic Impurities

• **RESIDUE ON IGNITION** (281): NMT 0.2%

• **CHLORIDE AND SULFATE**, Chloride (221)

Sample solution: Dissolve 1.0-g in 100 mL of 0.15 N nitric acid.

Acceptance criteria: Shows no more turbidity with 1 mL of silver nitrate TS than 1.4 mL of 0.020 N hydrochloric acid in 100 mL of 0.15 N nitric acid (0.1%)

### Organic Impurities

#### • PROCEDURE

Mobile phase: Prepare as directed in the *Assay*.

Sample solution 1: 0.1 mg/mL of Atenolol in *Mobile phase*

Sample solution 2: 0.5 µg/mL of Atenolol, from *Sample solution 1* in *Mobile phase*

Chromatographic system: Proceed as directed in the *Assay*, except use the injection size listed below.

Injection size: 50 µL

Analysis

Samples: *Sample solution 1* and *Sample solution 2*

[NOTE—Chromatograph *Sample solution 1* for a period of time that is 6 times the retention time of the atenolol peak.]

Calculate the percentage of each impurity in *Sample solution 1*:

$$\text{Result} = 0.5(r_U/r_A)$$

$r_U$  = peak response of any individual impurity in *Sample solution 1*

$r_A$  = peak response of Atenolol in *Sample solution 2*

Acceptance criteria

Individual impurities: NMT 0.25%

Total impurities: NMT 0.5%

## SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE**, Class I (741):

152°–156.5°

• **LOSS ON DRYING** (731): Dry a sample at 105° to constant weight; it loses NMT 0.5% of its weight.

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE**: Preserve in well-closed containers. Store at room temperature.

## • USP REFERENCE STANDARDS (11)

USP Atenolol RS

## Atenolol Injection

» Atenolol Injection is a sterile solution of Atenolol in Water for Injection. It contains a suitable buffering agent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of atenolol ( $C_{14}H_{22}N_2O_3$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, in a cool place or at controlled room temperature, protected from light. Avoid freezing.

**USP Reference standards** (11)—

USP Atenolol RS

USP Endotoxin RS

**Identification**—

**A:** The retention time of the main peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10 µg of atenolol per mL.

*Medium:* methanol.

**Bacterial Endotoxins Test** (85)—It contains not more than 33.3 USP Endotoxin Units per mg of atenolol.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.5 and 6.5.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Assay**—

*Citric acid buffer*—Transfer 2.5 g of citric acid to a 500-mL volumetric flask, add 400 mL of water, and swirl to dissolve. Adjust the solution with 2 N sodium hydroxide to a pH of 6.0, dilute with water to volume, and mix.

*Mobile phase*—Dissolve 930 mg of sodium octyl sulfate in 740 mL of water, add 8 mL of 3.6 N sulfuric acid, mix, and pass through a 1-µm or finer porosity filter. To the filtrate add 250 mL of acetonitrile, mix, and degas. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Standard preparation*—Transfer about 50 mg of USP Atenolol RS to a 100-mL volumetric flask, add 80 mL of *Citric acid buffer*, and sonicate for about 30 seconds to achieve dissolution. Dilute with *Citric acid buffer* to volume, and mix. Transfer 4.0 mL of this solution to a 10-mL volumetric flask, dilute with *Citric acid buffer* to volume, and mix. This solution contains about 0.2 mg of USP Atenolol RS per mL.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to 2 mg of atenolol, to a 10-mL volumetric flask, dilute with *Citric acid buffer* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2, and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into



the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $C_{14}H_{22}N_2O_3$  in each mL of the Injection taken by the formula:

$$10(C/V)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Atenolol RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the atenolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Atenolol Compounded Oral Solution

### DEFINITION

Atenolol Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of atenolol ( $C_{14}H_{22}N_2O_3$ ).

Prepare Atenolol Compounded Oral Solution at a 2-mg/mL concentration, for example, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Atenolol	200 mg
Glycerin	5 mL
Vehicle for Oral Suspension	45 mL
Vehicle for Oral Solution, Sugar Free, a sufficient quantity to make	100 mL

Calculate the quantity of each ingredient required for the total volume and atenolol strength to be prepared. Mix the *Atenolol*, previously pulverized, and *Glycerin* to form a smooth paste. Incorporate the *Vehicle for Oral Suspension* or an equal volume of *Vehicle for Oral Solution, Sugar Free*. [NOTE—The *Vehicle for Oral Suspension* may be omitted.] Incorporate sufficient *Vehicle for Oral Solution, Sugar Free* in increments to bring to volume, and mix well. [NOTE—Do not use a sucrose-containing vehicle for oral solution.] Package, and label.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in amber, tight containers, and store at controlled room temperature.
- **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded when stored at controlled room temperature
- **LABELING:** Label it to state that it is to be shaken well before use, and to state the *Beyond-Use Date*.

## Atenolol Tablets

### DEFINITION

Atenolol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of atenolol ( $C_{14}H_{22}N_2O_3$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Sample:** Mix a quantity of powdered Tablets, equivalent to 100 mg of atenolol, with 15 mL of methanol, heat the mixture to 50°, and shake for 5 min. Filter, and evaporate the filtrate on a water bath to dryness. Add 10 mL of 0.1 N hydrochloric acid to the residue, warm the solution, shake, and filter. To the filtrate add sufficient 1 N sodium hydroxide to make it alkaline, and extract the solution with 10 mL of chloroform, drying the chloroform extract over anhydrous sodium sulfate. Filter the dried chloroform solution, evaporate the fil-

trate on a water bath to dryness, and dry the residue at 105° for 1 h.

- **B.** The retention time of the atenolol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Mobile phase:** 1.1 g of sodium 1-heptanesulfonate and 0.71 g of anhydrous dibasic sodium phosphate in 700 mL of water. Add 2 mL of dibutylamine, and adjust with 0.8 M phosphoric acid to a pH of 3.0. Add 300 mL of methanol, and pass through a filter having a 0.5- $\mu$ m or finer porosity. Degas this solution before use.

**Standard solution:** 0.01 mg/mL of USP Atenolol RS in *Mobile phase*

**Sample stock solution:** Transfer 10 Tablets to a 1000-mL volumetric flask. Add 500 mL of *Mobile phase*, and sonicate for 15 min to disintegrate the Tablets. Dilute with *Mobile phase* to volume.

**Sample solution:** Centrifuge a portion of the *Sample stock solution*, and dilute a volume of the supernatant with *Mobile phase* to obtain a solution nominally containing 0.01 mg/mL of atenolol.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 226 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 0.6 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 5000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{14}H_{22}N_2O_3$  in each Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Atenolol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of atenolol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Medium:** 0.1 N acetate buffer, pH 4.6 (prepared by mixing 44.9 parts (v/v) of 0.1 N sodium acetate with 55.1 parts (v/v) of 0.1 N acetic acid solution, and adjust with either diluted sodium hydroxide or diluted acetic acid to a pH of 4.6); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

Determine the amount of  $C_{14}H_{22}N_2O_3$  dissolved by using the following method.

**Mobile phase, Chromatographic system, and System suitability:** Proceed as directed in the *Assay* under *Atenolol*.

**Standard solution:** 0.01 mg/mL of USP Atenolol RS in *Mobile phase*

**Sample solution:** Pass a portion of the solution under test through a suitable 0.45- $\mu$ m filter. Quantitatively dilute a measured volume of the filtrate with *Mobile phase* to obtain a solution estimated to contain about 0.01 mg/mL of atenolol.



**Analysis:** Proceed as directed in the Assay.  
Calculate the percentage of  $C_{14}H_{22}N_2O_3$  dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times D \times (100/L)$$

- $r_u$  = peak response from the *Sample solution*  
 $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Atenolol RS in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 900 mL  
 $D$  = dilution factor for the *Sample solution*  
 $L$  = Tablet label claim (mg)

**Tolerances:** NLT 80% (Q) of the labeled amount of  $C_{14}H_{22}N_2O_3$  is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP Atenolol RS

### Atenolol Compounded Oral Suspension

#### DEFINITION

Atenolol Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of atenolol ( $C_{14}H_{22}N_2O_3$ ).

Prepare Atenolol Compounded Oral Suspension 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Atenolol powder	1 g
Vehicle: a 1:1 mixture of Ora-Plus <sup>a</sup> and Ora-Sweet SF <sup>a</sup> , a sufficient quantity to make	100 mL

<sup>a</sup> Perrigo Pharmaceuticals, Allegan, MI.

Pour *Atenolol powder* into a suitable container. Wet the powder with a small amount of *Vehicle*, and triturate to make a smooth paste. Add *Vehicle* to make the contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the remainder of *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

#### ASSAY

##### • PROCEDURE

**Solution A:** 25 mM sodium phosphate adjusted with phosphoric acid to a pH of 3.0

**Solution B:** Water adjusted with phosphoric acid to a pH of 3.0

**Solution C:** Methanol and *Solution B* (50:50)

**Mobile phase:** Acetonitrile and *Solution A* (15:85). Filter, and degas.

**Standard stock solution:** 10 mg/mL of USP Atenolol RS in *Solution C*. Mix well and sonicate for 3 min. Store at 2°–8°.

**Standard solution:** Transfer 2.0 mL of the *Standard stock solution* to a 500-mL volumetric flask, add 0.5 mL of *Solution C*, and dilute with *Solution B* to volume. Mix well. Centrifuge a portion of the resultant solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

**Sample solution:** Shake each bottle of Oral Suspension thoroughly. Transfer 2.0 mL of Oral Suspension into a 500-mL volumetric flask, and add 0.5 mL of *Solution C*.

Dilute with *Solution B* to volume. Mix well. Centrifuge a portion of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 227 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Temperatures**

**Column:** 30°

**Autosampler:** 5°

**Flow rate:** 0.7 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for atenolol is about 5.1 min.]

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of atenolol ( $C_{14}H_{22}N_2O_3$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of atenolol from the *Sample solution*  
 $r_s$  = peak response of atenolol from the *Standard solution*  
 $C_s$  = concentration of atenolol in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of atenolol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

- **pH** (791): 6.4–7.4

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or at controlled room temperature
- **USP REFERENCE STANDARDS** (11)  
USP Atenolol RS

### Atenolol Compounded Oral Suspension, Veterinary

#### DEFINITION

Atenolol Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of atenolol ( $C_{14}H_{22}N_2O_3$ ). Prepare Atenolol Compounded Oral Suspension, Veterinary 25 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Atenolol powder	2.5 g
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<sup>a</sup> Perrigo Pharmaceuticals, Allegan, MI.



Vehicle: a 1:1 mixture of Ora-Plus <sup>a</sup> and Ora-Sweet SF <sup>a</sup> , a sufficient quantity to make	100 mL
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<sup>a</sup>Perrigo Pharmaceuticals, Allegan, MI.

Pour the *Atenolol* powder into a suitable container. Wet the powder with a small amount of *Vehicle*, and triturate to make a smooth paste. Add the *Vehicle* to make the contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

## ASSAY

### PROCEDURE

**Solution A:** 25 mM sodium phosphate adjusted with phosphoric acid to a pH of 3.0

**Mobile phase:** Acetonitrile and *Solution A* (15:85). Filter, and degas.

**Solution B:** Water adjusted with phosphoric acid to a pH of 3.0

**Solution C:** Methanol and *Solution B* (50:50)

**Standard stock solution:** 25 mg/mL of USP Atenolol RS in *Solution C*. Mix well, and sonicate for 3 min. Store at 2°–8°.

**Standard solution:** Transfer 2.0 mL of the *Standard stock solution* to a 1-L volumetric flask, and add 0.5 mL of *Solution C*. Dilute with *Solution B* to volume, and mix well. Centrifuge a portion of the resultant solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

**Sample solution:** Shake thoroughly each bottle of Oral Suspension, Veterinary. Transfer 2.0 mL of Oral Suspension, Veterinary to a 1-L volumetric flask, and add 0.5 mL of *Solution C*. Dilute with *Solution B* to volume. Centrifuge a portion of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 227 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Temperatures**

**Column:** 30°

**Autosampler:** 5°

**Flow rate:** 0.7 mL/min

**Injection volume:** 20 μL

### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for atenolol is about 5.1 min.]

### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of atenolol (C<sub>14</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub>) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of atenolol from the *Sample solution*

$r_s$  = peak response of atenolol from the *Standard solution*

$C_s$  = concentration of atenolol in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of atenolol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

## SPECIFIC TESTS

• **pH (791):** 9.1–10.1

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.

• **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*. Label it to state that it is for veterinary use only.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or controlled room temperature

• **USP REFERENCE STANDARDS (11)**  
USP Atenolol RS

## Atenolol and Chlorthalidone Tablets

» Atenolol and Chlorthalidone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of atenolol (C<sub>14</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub>) and chlorthalidone (C<sub>14</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>4</sub>S).

**Packaging and storage—**Preserve in well-closed containers.

### USP Reference standards (11)—

USP Atenolol RS

USP Chlorthalidone RS

### Identification—

**A:** Shake a quantity of powdered Tablets, equivalent to about 50 mg of chlorthalidone, with 5 mL of methanol for 15 minutes, and filter. Apply 10 μL of this test solution, 10 μL of a *Standard solution* of USP Chlorthalidone RS in methanol containing 10 mg per mL, and 10 μL of a second *Standard solution* of USP Atenolol RS in methanol containing 10 mg per mL,  $I$  being the ratio of the labeled amount, in mg, of atenolol to the labeled amount, in mg, of chlorthalidone per Tablet to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-butyl alcohol and 1 N ammonium hydroxide (5:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and air-dry. Locate the spots on the plate by viewing under short-wavelength UV light: the principal spots obtained from the test solution correspond in  $R_f$  value, size, and intensity to those obtained from the respective *Standard solutions*.

**B:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

**Medium:** 0.01 N hydrochloric acid; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

Determine the amounts of atenolol (C<sub>14</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub>) and chlorthalidone (C<sub>14</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>4</sub>S) dissolved by employing the following method.

**Mobile phase and Chromatographic system—**Prepare as directed in the *Assay*.

**Diluent—**Prepare a mixture of 1000 mL of acetonitrile and 32 mL of 3.6 N sulfuric acid.

**Standard solvent—**Prepare a mixture of water and *Diluent* (750: 225).



**Standard solution**—Dissolve accurately weighed quantities of USP Atenolol RS and USP Chlorthalidone RS in *Standard solvent* to obtain a solution having known concentrations of about 0.00085L mg of USP Atenolol RS and 0.00085L' mg of USP Chlorthalidone RS per mL, L and L' being the labeled amounts, in mg, of atenolol and chlorthalidone, respectively, per Tablet.

**Test solution**—Mix 10.0 mL of the filtered solution under test and 3.0 mL of *Diluent*.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of atenolol ( $C_{14}H_{22}N_2O_3$ ) and chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) dissolved by the same formula:

$$1170C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate Reference Standard in the *Standard solution*; and  $r_U$  and  $r_S$  are the responses of the corresponding analyte obtained from the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 80% (Q) of the labeled amount of atenolol ( $C_{14}H_{22}N_2O_3$ ) is dissolved in 45 minutes, and not less than 70% (Q) of the labeled amount of chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Proceed as directed in the *Assay*, except to prepare the *Assay preparation* as follows. Transfer 1 Tablet to a volumetric flask of such capacity that when filled to volume, a concentration of about 0.25 mg of chlorthalidone per mL is obtained. Add a mixture of water and acetonitrile (1:1) to about half the capacity of the flask, and shake by mechanical means for not less than 15 minutes to disintegrate the Tablet. Dilute with water to volume, and mix. Pass a portion of this solution through a filter having a 0.5- $\mu$ m or finer porosity, and use the filtrate as the *Assay preparation*. Calculate the quantities, in mg, of atenolol ( $C_{14}H_{22}N_2O_3$ ) and chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) in the Tablet taken by the formula:

$$CV(r_U / r_S)$$

in which V is the volume, in mL, of the volumetric flask used to prepare the *Assay preparation*; and the other terms are as defined in the *Assay*.

#### Assay—

**Mobile phase**—Prepare a mixture of 740 mL of water, 250 mL of acetonitrile, 8 mL of 3.6 N sulfuric acid, and 930 mg of sodium octyl sulfate. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve accurately weighed quantities of USP Atenolol RS and USP Chlorthalidone RS in a mixture of water and acetonitrile (3:1) to obtain a solution having known concentrations of about 0.25 mg of USP Chlorthalidone RS and 0.25J mg of USP Atenolol RS per mL, J being the ratio of the labeled amount, in mg, of atenolol to the labeled amount, in mg, of chlorthalidone per Tablet.

**Assay preparation**—Transfer 10 Tablets to a volumetric flask of such capacity that when filled to volume, a concentration of about 0.5 mg of chlorthalidone per mL is obtained. Add a mixture of water and acetonitrile (1:1) to about half the capacity of the flask, and shake by mechanical means for not less than 15 minutes to disintegrate the Tablets. Dilute with a mixture of water and acetonitrile (1:1) to volume, and mix. Pass a portion of this stock solution through a filter having a 0.5- $\mu$ m or finer porosity. Transfer 25.0 mL of the clear filtrate to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 275-nm detector

and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.7 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 for atenolol and 1.0 for chlorthalidone; the resolution,  $R$ , between the atenolol and chlorthalidone peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

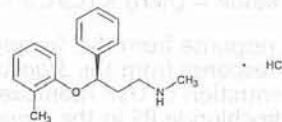
**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Assay preparation* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of atenolol ( $C_{14}H_{22}N_2O_3$ ) and chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) in each Tablet taken by the formula:

$$2C(V/10)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; V is the volume, in mL, of the volumetric flask used to prepare the stock solution for the *Assay preparation*; and  $r_U$  and  $r_S$  are the responses for the corresponding analyte obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**NOTE**—If a trailing peak or shoulder is observed on the chlorthalidone peak with a relative retention time of not more than 1.1 in the chromatograms of both the *Standard preparation* and the *Assay preparation*, sum the areas for the chlorthalidone peak with the trailing peak or shoulder to report the peak responses for chlorthalidone.

## Atomoxetine Hydrochloride



$C_{17}H_{21}NO \cdot HCl$  291.82  
Benzenepropanamine, N-methyl- $\gamma$ -(2-methylphenoxy)-, hydrochloride, (-);  
(-)-N-Methyl-3-phenyl-3-(o-tolyloxy)propylamine hydrochloride [82248-59-7].

#### DEFINITION

Atomoxetine Hydrochloride contains NLT 98.0% and NMT 102.0% of atomoxetine hydrochloride ( $C_{17}H_{21}NO \cdot HCl$ ), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the atomoxetine R-isomer from the *System suitability solution*, as obtained in the test for *Organic Impurities, Procedure 2*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements of the silver nitrate precipitate test

#### ASSAY

##### • PROCEDURE

**Buffer:** 2.9 g/L of phosphoric acid in water. Adjust with 5 M potassium hydroxide solution to a pH of 2.5. To 1 L of this solution add 5.9 g of octanesulfonic acid sodium salt monohydrate.

**Mobile phase:** n-Propanol and *Buffer* (27:73). [NOTE—The ratio of n-propanol in *Buffer* can be varied between 26:74 and 29:71 to meet system suitability requirements.]

**System suitability solution:** 0.1 mg/mL of USP Mandelic Acid RS, 0.15 mg/mL of USP Atomoxetine Related



Compound A RS, and 0.25 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase*

**Standard solution:** 0.25 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase*. Sonication may be used to aid in dissolution.

**Sample solution:** 0.25 mg/mL of Atomoxetine Hydrochloride in *Mobile phase*. Sonication may be used to aid in dissolution.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm × 15-cm; 3.5-μm packing L7

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**Run time:** 1.3 times the retention time of atomoxetine

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* in *Organic Impurities, Procedure 1* for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 5.0 between mandelic acid and atomoxetine related compound A, *System suitability solution*

**Tailing factor:** NMT 1.5 for atomoxetine, *System suitability solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of atomoxetine hydrochloride (C<sub>17</sub>H<sub>21</sub>NO · HCl) in the portion of Atomoxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Atomoxetine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Atomoxetine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

#### Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 10 ppm (Official 1-Jan-2018)

#### ORGANIC IMPURITIES

[NOTE—It is required to perform *Organic Impurities, Procedure 1* and *Organic Impurities, Procedure 2*.]

#### Procedure 1

**Buffer and Mobile phase:** Prepare as directed in the *Assay*.

**System suitability solution:** 0.10 mg/mL of USP Mandelic Acid RS, 0.15 mg/mL of USP Atomoxetine Related Compound A RS, and 0.25 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase*

**Standard solution:** 0.0025 mg/mL of USP Atomoxetine Hydrochloride RS in *Mobile phase*

**Sample solution:** 2.5 mg/mL of Atomoxetine Hydrochloride in *Mobile phase*

**Chromatographic system:** Proceed as directed in the *Assay*, except for *Run time*.

**Run time:** 2.6 times the retention time of atomoxetine

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 5.0 between mandelic acid and atomoxetine related compound A, *System suitability solution*

**Tailing factor:** NMT 1.5 for atomoxetine, *System suitability solution*

**Relative standard deviation:** NMT 5% from three injections, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Atomoxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of atomoxetine from the *Standard solution*

$C_S$  = concentration of USP Atomoxetine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Atomoxetine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Mandelic acid	0.20	0.10
Atomoxetine related compound A	0.27	0.10
Desmethyl atomoxetine <sup>a</sup>	0.73	0.3
Atomoxetine	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	0.5

<sup>a</sup> (R)-N-Methyl-3-phenoxy-3-phenylpropan-1-amine.

#### Procedure 2

**Mobile phase:** Isopropyl alcohol, diethylamine, tri-fluoroacetic acid, and *n*-hexane (150: 1.5: 2.0: 846.5)

**System suitability solution:** 3.5 mg/mL of USP Atomoxetine Hydrochloride RS, 17.5 μg/mL of USP Atomoxetine S-Isomer RS, and 3.5 μg/mL of USP Atomoxetine Related Compound B RS, prepared by first dissolving the Reference Standards in absolute alcohol, using 25% of the final volume. Dilute with *n*-hexane to volume.

**Sample solution:** 3.5 mg/mL of Atomoxetine Hydrochloride prepared by first dissolving it in absolute alcohol, using 25% of the final volume. Dilute with *n*-hexane to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 273 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L40

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**Run time:** 1.3 times the retention time of atomoxetine

#### System suitability

**Sample:** *System suitability solution*

[NOTE—See *Table 2* for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.75 between atomoxetine S-isomer and atomoxetine related compound B



**Tailing factor:** NMT 1.8 for atomoxetine

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of atomoxetine related compound B, atomoxetine related compound C, and atomoxetine *S*-isomer in the portion of Atomoxetine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_T$  = sum of all the peak responses of atomoxetine related compound B, atomoxetine related compound C, atomoxetine *S*-isomer, and atomoxetine from the *Sample solution*

**Acceptance criteria:** See Table 2.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Atomoxetine <i>S</i> -isomer <sup>a</sup>	0.47	0.5
Atomoxetine related compound C <sup>b</sup>	0.52	0.1
Atomoxetine related compound B	0.56	0.1
Atomoxetine	1.0	—

<sup>a</sup> *N*-Methyl-3-phenyl-3-(*o*-tolylloxy)propan-1-amine.

<sup>b</sup> *N*-Methyl-3-phenyl-3-(*p*-tolylloxy)propan-1-amine.

#### SPECIFIC TESTS

##### • LOSS ON DRYING (731)

**Analysis:** Dry under vacuum at 105° for 2 h.

**Acceptance criteria:** NMT 0.5%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at room temperature.

##### • USP REFERENCE STANDARDS (11)

USP Atomoxetine Hydrochloride RS

USP Atomoxetine Related Compound A RS

3-(Methylamino)-1-phenylpropan-1-ol.

$C_{10}H_{15}NO$  165.23

USP Atomoxetine Related Compound B RS

*N*-Methyl-3-phenyl-3-(*m*-tolylloxy)propan-1-amine hydrochloride.

$C_{17}H_{21}NO \cdot HCl$  291.82

USP Atomoxetine *S*-Isomer RS

(*S*)-*N*-Methyl-3-phenyl-3-(*o*-tolylloxy)propan-1-amine hydrochloride.

$C_{17}H_{21}NO \cdot HCl$  291.82

USP Mandelic Acid RS

$\alpha$ -Hydroxyphenylacetic acid.

$C_8H_8O_3$  152.15

## Atomoxetine Capsules

#### DEFINITION

Atomoxetine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of atomoxetine ( $C_{17}H_{21}NO$ ).

#### IDENTIFICATION

##### • A. INFRARED ABSORPTION (197K) or (197A)

**Standard:** 6 mg/mL of USP Atomoxetine Hydrochloride RS in methanol. Dry the solution to a dry powder under an air or nitrogen purge for a minimum of 3 h.

**Sample:** Shake the contents of a sufficient number of Capsules, equivalent to about 60 mg of atomoxetine, with 10 mL of methanol. Centrifuge at 4000 rpm for 5 min. Evaporate the solution to a dry powder with the aid of a current of air or stream of nitrogen.

**Acceptance criteria:** The IR spectrum exhibits main bands at ( $\pm 2$ ) wavenumbers ( $cm^{-1}$ ) 2955, 2855, 1599–1604, 1492, 1048, 1023, and 1010.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Buffer:** 5.8 g/L of monobasic potassium phosphate in water. To each liter of this solution add 3.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** Acetonitrile and Buffer (38:62)

**System suitability solution:** 0.1 mg/mL of atomoxetine (free base) from USP Atomoxetine Hydrochloride RS and 0.02 mg/mL of *o*-cresol in *Mobile phase*. Sonicate to aid in dissolution.

**Standard solution:** 0.1 mg/mL of atomoxetine (free base) from USP Atomoxetine Hydrochloride RS in *Mobile phase*. Sonicate to aid in dissolution.

**Sample stock solution:** From NLT 10 Capsules (including shells) prepared as follows. Add the intact Capsules to a suitable volumetric flask. Add *Mobile phase* to fill 65% of the final volume. Allow to stand for at least 10 min, then shake for 20 min. Dilute with *Mobile phase* to volume.

**Sample solution:** Nominally 0.1 mg/mL of atomoxetine, prepared by diluting a suitable volume of *Sample stock solution* with *Mobile phase*

##### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  7.5-cm; 3.5- $\mu$ m packing L7

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu$ L

**Run time:** 1.7 times the retention time of atomoxetine

##### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for atomoxetine and *o*-cresol are 1.0 and 1.3, respectively.]

##### Suitability requirements

**Resolution:** NLT 3.5 between atomoxetine and *o*-cresol, *System suitability solution*

**Tailing factor:** NMT 2.0 for atomoxetine, *System suitability solution*

**Relative standard deviation:** NMT 1.0% for atomoxetine, *Standard solution*

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of atomoxetine ( $C_{17}H_{21}NO$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of atomoxetine in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of atomoxetine in the *Sample solution* (mg/mL)



Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 1000 mL, deaerated

Apparatus 2: 50 rpm, with three-prong sinker

Time: 30 min

Buffer and Mobile phase: Prepare as directed in the Assay.

Standard stock solution: 0.1 mg/mL of atomoxetine (free base) from USP Atomoxetine Hydrochloride RS in Medium. Sonicate to aid in dissolution.

Standard solution: Dilute the Standard stock solution with Medium to obtain a final concentration of (L/1000) mg/mL, where L is the Capsule label claim in mg.

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system: Proceed as directed in the Assay.

### System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.4%

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of atomoxetine (C<sub>17</sub>H<sub>21</sub>NO) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of atomoxetine in the Standard solution (mg/mL)

L = label claim (mg/Capsule)

V = volume of Medium (mL)

Tolerances: NLT 80% (Q) of the labeled amount of atomoxetine (C<sub>17</sub>H<sub>21</sub>NO) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

Buffer: Dissolve 4.9 g of sodium 1-decanesulfonate and 6.9 g of monobasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 3.1.

Mobile phase: Acetonitrile and Buffer (41:59)

Sensitivity solution: 0.1 µg/mL of atomoxetine in Mobile phase

System suitability solution: 1 mg/mL of atomoxetine containing atomoxetine N-amide prepared as follows. Weigh equal amounts of USP Atomoxetine Hydrochloride RS and urea, and place in a volumetric flask. Add water to fill 10% of the final volume. Sonicate for 3 min. Place the flask in an 85° oven for 40 min. Allow the solution to cool to room temperature. Dilute with Mobile phase to volume. [NOTE—The oven temperature and time in the oven can be adjusted to give a suitable level of atomoxetine N-amide peak.]

Sample solution: 1 mg/mL of atomoxetine in Mobile phase, from the contents of NLT 5 Capsules prepared as follows. Transfer the Capsule contents to a suitable volumetric flask. Fill 50% of the final volume with Mobile phase. Swirl, and let stand for 15 min. Dilute with Mobile phase to volume.

### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 µL

Run time: 2.3 times the retention time of atomoxetine

### System suitability

Samples: Sensitivity solution and System suitability solution

[NOTE—See Table 1 for the relative retention times.]

### Suitability requirements

Resolution: NLT 2.6 between atomoxetine and atomoxetine N-amide, System suitability solution

Relative standard deviation: NMT 5%, Sensitivity solution

### Analysis

Sample: Sample solution

Calculate the percentage of each individual impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each individual impurity from the Sample solution

$r_T$  = sum of all the peak responses from the Sample solution

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Desmethyl atomoxetine <sup>a</sup>	0.76	0.3
Atomoxetine	1.0	—
Atomoxetine N-amide <sup>b</sup>	1.2	0.2
Any individual unspecified degradation product	—	0.2
Total impurities	—	1.0

<sup>a</sup> (R)-N-Methyl-3-phenoxy-3-phenylpropan-1-amine.

<sup>b</sup> (R)-1-Methyl-1-[3-phenyl-3-(o-tolyloxy)propyl]urea.

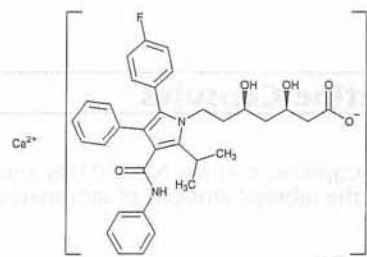
## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Atomoxetine Hydrochloride RS

## Atorvastatin Calcium



C<sub>66</sub>H<sub>68</sub>CaF<sub>2</sub>N<sub>4</sub>O<sub>10</sub>

1155.36

1H-Pyrrole-1-heptanoic acid, 2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylami-



no)carbonyl]-, calcium salt (2:1), [*R*-(*R*\*,*R*\*)]-; Calcium ( $\beta R, \delta R$ )-2-(*p*-fluorophenyl)- $\beta, \delta$ -dihydroxy-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)pyrrole-1-heptanoate (1:2); [(3*R*,5*R*)-7-[3-(Phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt] Anhydrous [134523-03-8].

$C_{66}H_{68}CaF_2N_4O_{10} \cdot 3H_2O$  1209.41  
Trihydrate [344423-98-9].

$C_{66}H_{68}CaF_2N_4O_{10} \cdot C_3H_8O_2$  1231.46  
Propylene glycol solvate

### DEFINITION

Atorvastatin Calcium contains NLT 98.0% and NMT 102.0% of atorvastatin calcium ( $C_{66}H_{68}CaF_2N_4O_{10}$ ), calculated on the anhydrous and solvent-free basis. If labeled as a propylene glycol solvate, it contains NLT 98.0% and NMT 102.0% of atorvastatin calcium ( $C_{66}H_{68}CaF_2N_4O_{10}$ ), calculated on the anhydrous, propylene glycol-free, and solvent-free basis. It may contain a suitable antioxidant.

### IDENTIFICATION

- A. INFRARED ABSORPTION (197K):** [NOTE—If a difference appears in the IR spectra of the analyte and the standard, separately dissolve equal portions of the sample specimen and the USP Reference Standard in equal volumes of methanol, evaporate the solution to dryness in similar containers under identical conditions, and repeat the test on the residues.]

- B. CALCIUM**

**Diluent:** Methanol, water, and hydrochloric acid (75:25:2)

**Sample solution:** 0.05 mg/mL of Atorvastatin Calcium in Diluent

**Blank:** Diluent

**Analysis**

**Samples:** Sample solution and Blank

**Instrumental conditions**

(See Atomic Absorption Spectroscopy (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** Calcium emission line at 422.7 nm

**Flame:** Air-acetylene

**Acceptance criteria:** The Sample solution exhibits a significant absorption at the calcium emission line at 422.7 nm.

### ASSAY

- PROCEDURE**

**Buffer:** 3.9 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of  $5.0 \pm 0.1$ .

**Solution A:** Acetonitrile, stabilizer-free tetrahydrofuran, and Buffer (21:12:67)

**Solution B:** Acetonitrile, stabilizer-free tetrahydrofuran, and Buffer (61:12:27)

**Mobile phase:** See Table 1. [NOTE—If necessary, adjust the Mobile phase by increasing or decreasing the percentage of acetonitrile or the pH of the ammonium acetate solution to achieve a retention time of 26–34 min for the atorvastatin peak.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	100	0
70	20	80

Table 1 (Continued)

Time (min)	Solution A (%)	Solution B (%)
85	0	100
100	0	100
105	100	0
115	100	0

**Diluent:** *N,N*-dimethylformamide

**System suitability solution:** 0.05 mg/mL of USP

Atorvastatin Calcium RS and 0.06 mg/mL of USP

Atorvastatin Related Compound B RS in Diluent

**Standard solution:** 0.4 mg/mL of USP Atorvastatin Calcium RS in Diluent. [NOTE—Use sonication if necessary.]

**Sample solution:** 0.4 mg/mL of Atorvastatin Calcium in Diluent. [NOTE—Use sonication if necessary.]

**Chromatographic system**

(See Chromatography (621), System Suitability.)

[NOTE—If significant fronting of the peaks for atorvastatin related compound B and atorvastatin is observed, use the following diluent to prepare the Sample solution, the Standard solution, and the System suitability solution: acetonitrile, stabilizer-free tetrahydrofuran, and water (1:1:2).]

**Mode:** LC

**Detector:** UV 244 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Samples:** System suitability solution and Standard solution

**Suitability requirements**

**Resolution:** NLT 1.5 between the peaks for atorvastatin related compound B and atorvastatin, System suitability solution

**Tailing factor:** NMT 1.6, Standard solution

**Relative standard deviation:** NMT 0.6%, Standard solution

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of atorvastatin calcium ( $C_{66}H_{68}CaF_2N_4O_{10}$ ) in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Atorvastatin Calcium RS in the Standard solution (mg/mL)

$C_U$  = concentration of Atorvastatin Calcium in the Sample solution (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis. If labeled as a propylene glycol solvate, 98.0%–102.0% on the anhydrous, propylene glycol-free, and solvent-free basis.

### OTHER COMPONENTS

- CONTENT OF PROPYLENE GLYCOL** (if labeled as a propylene glycol solvate)

**Diluent:** Dimethylsulfoxide

**Standard solution:** 0.125 mg/mL of propylene glycol in Diluent

**Sample solution:** 2.5 mg/mL of Atorvastatin Calcium (as propylene glycol solvate) in Diluent. Use sonication as needed to achieve a complete dissolution.

**Chromatographic system**

(See Chromatography (621), System Suitability.)



Mode: GC  
 Detector: Flame ionization  
 Column: 0.53-mm × 75-m; 3-μm coating of G43  
 Temperatures  
 Injection port: 230°  
 Detector: 250°  
 Column: See Table 2.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	0	100	1
100	10	140	5
140	30	225	3

Carrier gas: Helium  
 Flow rate: 6.0 mL/min  
 Injection volume: 1 μL  
 Injection type: Splitless, using a suitable inlet liner  
 System suitability  
 Sample: *Standard solution*  
 Suitability requirements  
 Tailing factor: NMT 2.0  
 Relative standard deviation: NMT 5.0%

**Analysis**

Samples: *Standard solution* and *Sample solution*  
 Calculate the percentage of propylene glycol in the portion of Atorvastatin Calcium as propylene glycol solvate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of propylene glycol from the *Sample solution*  
 $r_S$  = peak response of propylene glycol from the *Standard solution*  
 $C_S$  = concentration of propylene glycol in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Atorvastatin Calcium (as propylene glycol solvate) in the *Sample solution* (mg/mL)

Acceptance criteria: 5.4%–7.3%

**IMPURITIES****Delete the following:****• HEAVY METALS**

Diluent: Methanol and water (9:1)

Sample solution: Dissolve 250 mg of the sample in 30 mL of *Diluent*.

Standard lead solution: Prepare as directed in *Heavy Metals* (231).

Reference solution: Dilute 0.5 mL of the *Standard lead solution* with *Diluent* to 30 mL.

Blank solution: 20 mL of *Diluent*

Monitor solution: Dissolve 250 mg of Atorvastatin Calcium in 0.5 mL of the *Standard lead solution*, and dilute with *Diluent* to 30 mL.

**Analysis**

Samples: *Sample solution*, *Reference solution*, *Blank solution*, and *Monitor solution*

To each solution, add 2 mL of pH 3.5 Acetate Buffer prepared as directed in *Heavy Metals* (231). Mix, add to 1.2 mL of thioacetamide–glycerin base TS, and mix

immediately. Pass the solutions through a membrane filter of 0.45-μm pore size. Compare the spots on the filters obtained with the different solutions. The brown color of the spot from the *Sample solution* is not more intense than that of the spot from the *Reference solution*. The test is invalid if the *Reference solution* does not show a slight brown color compared to the *Blank solution*, or if the color of the *Monitor solution* is not at least as intense as the color of the *Reference solution*.

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

- **ORGANIC IMPURITIES, PROCEDURE 1:** [NOTE—On the basis of the synthetic route or of the solid state nature of the drug substance, perform either *Procedure 1* or *Procedure 2*. *Procedure 2* may be suitable when atorvastatin lactone, atorvastatin epoxy tetrahydrofuran analog, and atorvastatin acetonide are possible related compounds, and it may be suitable for an amorphous form of the drug substance.]

Buffer, *Solution A*, *Solution B*, *Mobile phase*, *Diluent*, *System suitability solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.

Standard solution: 1.5 μg/mL each of USP Atorvastatin Related Compound A RS, USP Atorvastatin Related Compound B RS, USP Atorvastatin Related Compound C RS, and USP Atorvastatin Related Compound D RS in *Diluent*

Sample solution: 1 mg/mL of Atorvastatin Calcium in *Diluent*. [NOTE—Use sonication if necessary.]

**System suitability**

Sample: *System suitability solution*

**Suitability requirements**

Resolution: NLT 1.5 between the peaks for atorvastatin related compound B and atorvastatin

**Analysis**

Samples: *Standard solution* and *Sample solution*  
 Chromatograph the *Standard solution*, and identify the components based on their relative retention times, given in *Table 3*.

Calculate the percentage of each of the atorvastatin related compounds A, B, C, and D in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the relevant atorvastatin related compound from the *Sample solution*  
 $r_S$  = peak response of the relevant atorvastatin related compound from the *Standard solution*  
 $C_S$  = concentration of the relevant atorvastatin related compound in the *Standard solution* (mg/mL)

$C_U$  = concentration of Atorvastatin Calcium in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of any other individual impurity from the *Sample solution*

$r_T$  = sum of all the peak responses from the *Sample solution*

Acceptance criteria: See *Table 3*. Disregard any peak observed in the blank; the reporting level for impurities is 0.05%.



Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Atorvastatin related compound A <sup>a</sup>	0.8	0.3
Atorvastatin related compound B <sup>b</sup>	0.9	0.3
Atorvastatin	1.0	—
Atorvastatin related compound C <sup>c</sup>	1.2	0.3
Atorvastatin related compound D <sup>d,e</sup>	2.1	0.2
Any other individual impurity	—	0.1
Total impurities <sup>f</sup>	—	1.0

<sup>a</sup> Desfluoro impurity.<sup>b</sup> 3S,5R Isomer.<sup>c</sup> Difluoro impurity.<sup>d</sup> Epoxide impurity.<sup>e</sup> Atorvastatin related compound D may undergo a conversion to its cyclic hemiketal, which is a specified impurity listed in Table 5 in *Organic Impurities, Procedure 2*, as "atorvastatin epoxy tetrahydrofuran analog". The cyclic hemiketal of atorvastatin related compound D elutes about 1–2 min before atorvastatin related compound D. Use the sum of the areas of the two peaks as a peak response for atorvastatin related compound D in the *Standard solution* and the *Sample solution*.<sup>f</sup> This total does not include atorvastatin related compound E, as determined in the *Enantiomeric Purity* test.**• ORGANIC IMPURITIES, PROCEDURE 2**

**Buffer:** pH 5.0 mixture of 0.045 M ammonium formate and 0.0045 M ammonium acetate solutions, prepared as follows. Weigh 2.84 g of ammonium formate and 0.35 g of ammonium acetate, and dissolve in 950 mL of water. Adjust with 20% formic acid to a pH of 5.0, and dilute with water to 1 L.

**Solution A:** Acetonitrile and *Buffer* (33:67)

**Solution B:** Acetonitrile

**Solution C:** Stabilizer-free tetrahydrofuran

**Mobile phase:** See Table 4. Return to original conditions, and re-equilibrate the system.

Table 4

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	91	0	9
15	91	6	3
20	82	16	2
25	82	16	2
50	32	66	2
55	32	66	2

**Diluent:** Acetonitrile, stabilizer-free tetrahydrofuran, and *Buffer* (60:5:35)

**Peak identification solution:** 0.5 mg/mL of USP Atorvastatin Calcium RS and 2.5 µg/mL each of USP Atorvastatin Related Compound A RS, USP Atorvastatin Related Compound B RS, USP Atorvastatin Related Compound H RS, and USP Atorvastatin Related Compound I RS in *Diluent*

**Sample solution:** 0.5 mg/mL of Atorvastatin Calcium in *Diluent*. Use sonication to dissolve. [NOTE—The solution is stable for 3 h at room temperature and for 24 h when stored at 2°–8°, protected from light.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 4-µm packing L11

**Temperatures**

**Column:** 40°

**Autosampler:** 4°

**Flow rate:** 1.1 mL/min

**Injection volume:** 15 µL

**System suitability**

**Sample:** *Peak identification solution*

**Suitability requirements**

**Peak-to-valley ratio:** NLT 2 between the peaks for atorvastatin related compound B and atorvastatin

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_T) \times (1/F) \times 100$$

$r_U$  = peak response of the impurity from the *Sample solution*

$r_T$  = sum of all the peak responses, each divided by the corresponding value of the relative response factor from Table 5

$F$  = relative response factor for the impurity (see Table 5)

**Acceptance criteria:** See Table 5. Disregard any peak eluting before 2 min and any peak observed in the blank; the reporting level for impurities is 0.05%.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Atorvastatin diamino <sup>a</sup>	0.58	0.74	0.15
Atorvastatin related compound A <sup>b</sup>	0.86	1.0	0.3
Atorvastatin related compound B <sup>c</sup>	0.94	1.0	0.3
Atorvastatin	1.0	—	—
Atorvastatin related compound C <sup>d</sup> (if present)	1.1	1.0	0.3
Atorvastatin 3-deoxyhept-2-enoic acid <sup>e</sup>	1.45	1.0	0.10
Atorvastatin related compound H <sup>f</sup>	1.90	1.0	0.15
Atorvastatin epoxy tetrahydrofuran analog <sup>g</sup>	2.00	0.71	0.15
Atorvastatin ethyl ester <sup>h</sup>	2.08	1.0	0.15
Atorvastatin related compound D <sup>i</sup>	2.18	1.3	0.15

<sup>a</sup> (3R,5R)-7-((3R,5R)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanamido)-3,5-dihydroxyheptanoic acid.

<sup>b</sup> Desfluoro impurity.

<sup>c</sup> 3S,5R Isomer.

<sup>d</sup> Difluoro impurity.

<sup>e</sup> (5E)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl]-5-hydroxyhept-2-enoic acid.

<sup>f</sup> Lactone impurity.

<sup>g</sup> 4-(4-Fluorophenyl)-2,4-dihydroxy-2-isopropyl-N,5-diphenyl-3,6-dioxabicyclo[3.1.0]hexane-1-carboxamide.

<sup>h</sup> (3R,5R)-Ethyl 7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl)-3,5-dihydroxyheptanoate.

<sup>i</sup> Epoxide impurity.

<sup>j</sup> Acetonide impurity.

<sup>k</sup> This total does not include atorvastatin related compound E, as determined in the *Enantiomeric Purity* test.



Table 5 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Atorvastatin related compound II	2.75	1.0	0.15
Any other individual impurity	—	1.0	0.10
Total impurities <sup>a</sup>	—	—	1.0

<sup>a</sup> (3*R*,5*R*)-7-[(3*R*,5*R*)-7-[2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanamido]-3,5-dihydroxyheptanoic acid.

<sup>b</sup> Desfluoro impurity.

<sup>c</sup> 3*S*,5*R* Isomer.

<sup>d</sup> Difluoro impurity.

<sup>e</sup> (5*E*)-7-[2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]-5-hydroxyhept-2-enoic acid.

<sup>f</sup> Lactone impurity.

<sup>g</sup> 4-(4-fluorophenyl)-2,4-dihydroxy-2-isopropyl-*N*,5-diphenyl-3,6-dioxabicyclo[3.1.0]hexane-1-carboxamide.

<sup>h</sup> (3*R*,5*R*)-Ethyl 7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl)-3,5-dihydroxyheptanoate.

<sup>i</sup> Epoxide impurity.

<sup>j</sup> Acetonide impurity.

<sup>k</sup> This total does not include atorvastatin related compound E, as determined in the *Enantiomeric Purity* test.

#### • ENANTIOMERIC PURITY

**Mobile phase:** Hexane, dehydrated alcohol, and trifluoroacetic acid (940:60:1)

**System suitability stock solution:** 5 mg/mL of USP Atorvastatin Calcium RS and 37.5 µg/mL of USP Atorvastatin Related Compound E RS in methanol. [NOTE—Atorvastatin related compound E is the 3*S*,5*S* enantiomer of atorvastatin.]

**System suitability solution:** Transfer 2.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume.

**Sample solution:** Transfer 10 mg of Atorvastatin Calcium to a 10-mL volumetric flask, dissolve in 2.0 mL of methanol, add 2.0 mL of dehydrated alcohol, and dilute with hexane to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 244 nm

**Column:** 4.6-mm × 25-cm; packing L51

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The elution order of the peaks is atorvastatin related compound E followed by atorvastatin.]

**Resolution:** NLT 2.0 between the peaks for atorvastatin related compound E and atorvastatin

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of atorvastatin related compound E in the portion of Atorvastatin Calcium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for atorvastatin related compound E

$r_T$  = sum of the peak responses for atorvastatin related compound E and atorvastatin

**Acceptance criteria:** NMT 0.3% of atorvastatin related compound E

#### SPECIFIC TESTS

- **WATER DETERMINATION, Method Ia (921):** 3.5%–5.5% for the trihydrate form. If labeled as amorphous or as semicrystalline, NMT 6.0%. If labeled as a propylene glycol solvate, NMT 1.0%.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve the trihydrate form in well-closed containers, and store at room temperature. If labeled as amorphous or semicrystalline or as a propylene glycol solvate, store as per labeling instructions. Possible packaging and storage conditions could include the following: Preserve in well-closed containers protected from light and moisture, or in tight containers; store at room temperature, at controlled room temperature, or at 2°–8°; store under nitrogen atmosphere or packed with an oxygen absorber; and store under nitrogen atmosphere, packed with silica gel and an oxygen absorber.

- **LABELING:** Where it is an amorphous form, the label so indicates. Where it is a semicrystalline form, the label so indicates. Where it is a propylene glycol solvate form, the label so indicates. If a test for *Organic Impurities* other than *Procedure 1* is used, the labeling states the test with which the article complies. Label it to indicate the name and quantity of any added antioxidant.

#### • USP REFERENCE STANDARDS (11)

USP Atorvastatin Calcium RS

USP Atorvastatin Related Compound A RS

Desfluoro impurity, or (3*R*,5*R*)-7-[3-(phenylcarbamoyl)-2-isopropyl-4,5-diphenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.

$C_{66}H_{70}CaN_4O_{10}$  1119.38

USP Atorvastatin Related Compound B RS

3*S*,5*R* Isomer, or (3*S*,5*R*)-7-[3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.

$C_{66}H_{68}CaF_2N_4O_{10}$  1155.34

USP Atorvastatin Related Compound C RS

Difluoro impurity, or (3*R*,5*R*)-7-[3-(phenylcarbamoyl)-4,5-bis(4-fluorophenyl)-2-isopropyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.

$C_{66}H_{66}F_4N_4O_{10}$  1191.34

USP Atorvastatin Related Compound D RS

Epoxide impurity, or 3-(4-fluorobenzoyl)-2-isobutryl-3-phenyl-oxirane-2-carboxylic acid phenylamide.

$C_{26}H_{22}FNO_4$  431.46

USP Atorvastatin Related Compound E RS

3*S*,5*S* Enantiomer, or (3*S*,5*S*)-7-[3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt.

$C_{66}H_{68}CaF_2N_4O_{10}$  1155.34

USP Atorvastatin Related Compound H RS (lactone impurity)

5-(4-fluorophenyl)-1-{2-[(2*R*,4*R*)-4-hydroxy-6-oxotetrahydro-2*H*-pyran-2-yl]ethyl}-2-isopropyl-*N*,4-diphenyl-1*H*-pyrrole-3-carboxamide.

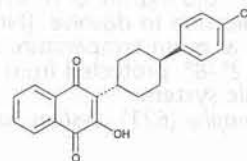
$C_{33}H_{33}FN_2O_4$  540.62

USP Atorvastatin Related Compound I RS (acetonide impurity)

*tert*-Butyl 2-((4*R*,6*R*)-6-{2-[2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl]ethyl}-2,2-dimethyl-1,3-dioxan-4-yl)acetate.

$C_{40}H_{47}FN_2O_5$  654.81

#### Atovaquone



$C_{22}H_{19}ClO_3$

1,4-Naphthalenedione, 2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-, *trans*;

366.84



2-[*trans*-4-(*p*-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone [95233-18-4].

### DEFINITION

Atovaquone contains NLT 97.5% and NMT 101.5% of atovaquone (C<sub>22</sub>H<sub>19</sub>ClO<sub>3</sub>), calculated on the anhydrous and organic solvent-free basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile, methanol, water, and phosphoric acid (525:175:300:5)

**Diluent:** Acetonitrile and water (80:20)

**System suitability solution:** 0.25 mg/mL of USP Atovaquone RS and 0.02 mg/mL of USP Atovaquone Related Compound A RS in *Diluent*. Store in a low-actinic glass container.

**Standard solution:** 0.25 mg/mL of USP Atovaquone RS in *Diluent*

**Sample solution:** 0.25 mg/mL of Atovaquone in *Diluent*. Use a low-actinic volumetric flask.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 3 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for atovaquone related compound A and atovaquone are about 0.85 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 4 between atovaquone related compound A and atovaquone, *System suitability solution*

**Column efficiency:** NLT 9000 theoretical plates, *Standard solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of atovaquone (C<sub>22</sub>H<sub>19</sub>ClO<sub>3</sub>) in the portion of Atovaquone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Atovaquone RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Atovaquone in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.5%–101.5% on the anhydrous and organic solvent-free basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

#### Delete the following:

- **HEAVY METALS** (231)

**Standard solution:** Add 1.0 mL of *Standard Lead Solution* to 0.5 g of magnesium oxide, and dry between

100° and 105°. Proceed as directed in the *Test preparation*, beginning with "Ignite to dull redness".

**Test preparation:** Mix 1.0 g of Atovaquone with 0.5 g of magnesium oxide thoroughly in a silica crucible. Ignite to dull redness until a homogeneous white or grayish-white mass is obtained. If the mixture remains colored after 30 min, allow to cool, mix using a fine glass rod, and repeat the ignition. If necessary, repeat the operation. Heat the residue at 800° for about 1 h. Cool, take up the residue in two 5-mL portions of 6 N hydrochloric acid, add 0.1 mL of phenolphthalein TS, and then add 13.5 N ammonium hydroxide until a pink color is obtained. Cool, add glacial acetic acid until the solution is decolorized, and add 0.5 mL in excess. Filter, if necessary, and wash the filter with water. Dilute with water to 20 mL.

**Blank solution:** Proceed as directed in the *Test preparation*, omitting the Atovaquone.

#### Analysis

**Samples:** *Standard solution*, *Test preparation*, and *Blank solution*

Transfer 12.0 mL of the *Test preparation* to a 50-mL color-comparison tube, 10.0 mL of the *Standard solution* to another, and 10.0 mL of the *Blank solution* to a third. Then add 2.0 mL of the *Test preparation* to the *Standard solution* as well as the *Blank solution*. Add 2 mL of pH 3.5 *Acetate Buffer* to each of the three tubes. Add 1.2 mL of thioacetamide–glycerin base TS. Allow to stand for 2 min, and view downward over a white surface.

**Acceptance criteria:** NMT 10 µg/g; the *Standard solution* is slightly brown when compared with the *Blank solution*, and the color of the *Test preparation* is not darker than the *Standard solution*. (Official 1-Jan-2018)

#### • RELATED COMPOUNDS

**System suitability solution and Sample solution:** Prepare as directed in the *Assay*.

#### Analysis

**Samples:** *System suitability solution* and *Sample solution*  
Using the chromatograms of the *Sample solution* and the *System suitability solution*, calculate the percentage of atovaquone related compounds in the portion of Atovaquone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_T$  = sum of all the peak responses from the *Sample solution*

**Acceptance criteria:** See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Indene isomer <sup>a</sup>	0.63	0.5
Atovaquone related compound A	0.85	1.0
Didehydroatovaquone <sup>b</sup>	0.89	0.3
Atovaquone	1.0	—
O-Methyl atovaquone <sup>c</sup>	1.8	0.5
Any other individual, unidentified impurity	—	0.2

<sup>a</sup> *trans*-2-[4-(4-Chlorophenyl)cyclohexyl]-1-oxo-1*H*-indene-3-carboxylic acid.

<sup>b</sup> (R*S*)-2-[4-(4-Chlorophenyl)cyclohex-3-enyl]-3-hydroxy-1,4-naphthoquinone.

<sup>c</sup> *trans*-2-[4-(4-Chlorophenyl)cyclohexyl]-3-methoxy-1,4-naphthoquinone.



Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sum of all other individual impurities	—	1.0
Total impurities	—	1.5

<sup>a</sup> *trans*-2-[4-(4-Chlorophenyl)cyclohexyl]-1-oxo-1*H*-indene-3-carboxylic acid.

<sup>b</sup> (R*S*)-2-[4-(4-Chlorophenyl)cyclohex-3-enyl]-3-hydroxy-1,4-naphthoquinone.

<sup>c</sup> *trans*-2-[4-(4-Chlorophenyl)cyclohexyl]-3-methoxy-1,4-naphthoquinone.

### SPECIFIC TESTS

- **WATER DETERMINATION**, Method I (921): NMT 1.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
  - USP Atovaquone RS
  - USP Atovaquone Related Compound A RS
  - cis*-2-[4-(4-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone.
  - C<sub>22</sub>H<sub>19</sub>ClO<sub>3</sub> 366.84

## Atovaquone Oral Suspension

### DEFINITION

Atovaquone Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of atovaquone (C<sub>22</sub>H<sub>19</sub>ClO<sub>3</sub>).

### IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION** (197U)
  - Medium**: Methanol and water (1:1)
  - Standard solution**: Dilute 5 mL of *Standard solution* from the *Assay* with *Medium* to 50 mL.
  - Sample solution**: Dilute 5 mL of *Sample solution* from the *Assay* with *Medium* to 50 mL.
  - Acceptance criteria**: Meets the requirements
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase**: Acetonitrile, methanol, water, and phosphoric acid (480:160:360:5)

**System suitability solution**: 0.09 mg/mL of USP Atovaquone RS and 0.01 mg/mL of USP Atovaquone Related Compound A RS in 0.1 M methanolic sodium hydroxide. Store in a low-actinic glass container.

**Standard stock solution**: 3 mg/mL of USP Atovaquone RS in a low-actinic, appropriately sized volumetric flask. Add 20% water and 60% 0.1 M methanolic sodium hydroxide. Sonicate for 5 min or until the material has dissolved. Allow to cool, and dilute with 0.1 M methanolic sodium hydroxide to volume.

**Standard solution**: 0.09 mg/mL of USP Atovaquone RS from *Standard stock solution*. Transfer to an appropriately sized, low-actinic volumetric flask in a mixture of methanol and water (1:1). Minimize exposure of this solution to light.

**Sample stock solution**: Nominally 3 mg/mL from a known volume of well-mixed Oral Suspension NLT 750 mg of atovaquone prepared as follows. In an appropriately sized, low-actinic volumetric flask, add 20% volume of water, swirl for 5 min, add 60% volume of 0.1 M methanolic sodium hydroxide, and sonicate for

15 min. Allow to cool, and dilute with 0.1 M methanolic sodium hydroxide to volume. Immediately filter a 20-mL portion, discarding the first 5 mL of the filtrate.

**Sample solution**: 0.09 mg/mL of atovaquone from the clear filtrate of the *Sample stock solution*. Transfer to an appropriately sized, low-actinic volumetric flask, and dilute with a mixture of methanol and water (1:1) to volume. Minimize exposure of this solution to light.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode**: LC

**Detector**: UV 220 nm

**Column**: 4.6-mm × 12.5-cm; packing L1

**Flow rate**: 3 mL/min

**Injection volume**: 20 µL

### System suitability

**Samples**: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for atovaquone related compound A and atovaquone are 0.86 and 1.0, respectively.]

### Suitability requirements

**Tailing factor**: NMT 1.5

**Relative standard deviation**: NMT 2.0%

### Analysis

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of atovaquone (C<sub>22</sub>H<sub>19</sub>ClO<sub>3</sub>) in the portion of Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

*r<sub>u</sub>* = peak response of atovaquone from the *Sample solution*

*r<sub>s</sub>* = peak response of atovaquone from the *Standard solution*

*C<sub>s</sub>* = concentration of USP Atovaquone RS in the *Standard solution* (mg/mL)

*C<sub>u</sub>* = nominal concentration of atovaquone in the *Sample solution* (mg/mL)

**Acceptance criteria**: 90.0%–110.0%

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements for oral suspension packaged in single-unit containers
- **DELIVERABLE VOLUME** (698): Meets the requirements for oral suspension packaged in multiple-unit containers

### IMPURITIES

#### ORGANIC IMPURITIES

**Mobile phase**, **System suitability solution**, **Standard solution**, **Sample solution**, **Chromatographic system**, and **System suitability**: Proceed as directed in the *Assay*.

#### Analysis

**Samples**: *System suitability solution*, *Standard solution*, and *Sample solution*

Using the chromatograms of the *System suitability solution* and the *Sample solution*, calculate the percentage of atovaquone related compounds in the portion of Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

*r<sub>u</sub>* = individual peak response of an atovaquone related compound, if any, from the *Sample solution*

*r<sub>s</sub>* = peak response of atovaquone from the *Standard solution*

*C<sub>s</sub>* = concentration of USP Atovaquone RS in the *Standard solution* (mg/mL)

*C<sub>u</sub>* = nominal concentration of Oral Suspension in the *Sample solution* (mg/mL)



*F* = relative response factor of an individual atovaquone related compound relative to the response of atovaquone (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Photodegradation peak <sup>a</sup>	0.3	—	—
Atovaquone impurity	0.65	1.08	0.5
Atovaquone related compound A	0.86	0.85	1.0
Atovaquone impurity	0.88	1.0	0.3
Atovaquone	1.0	1.0	—
Any other atovaquone related compound	—	1.0	0.2
Total impurities	—	—	2.0

<sup>a</sup> Disregard any peak having a relative retention time of 0.3, which is due to photodegradation during preparation of the Sample solution.

### SPECIFIC TESTS

• **pH (791):** 3.5–7.0

### • SEDIMENTATION

For oral suspension packaged in multiple-unit containers

**Analysis:** Transfer 50 mL of well-mixed Oral Suspension to a glass-stoppered graduated cylinder, and allow to stand for 16 h. Measure the volume, if any, of clear liquid observed in the cylinder.

**Acceptance criteria:** NMT 1 mL of clear liquid

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

### • USP REFERENCE STANDARDS (11)

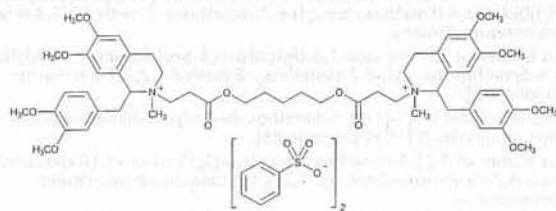
USP Atovaquone RS

USP Atovaquone Related Compound A RS

*cis*-2-[4-(4-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone.

C<sub>22</sub>H<sub>19</sub>ClO<sub>3</sub> 366.84

## Atracurium Besylate



C<sub>65</sub>H<sub>82</sub>N<sub>2</sub>O<sub>18</sub>S<sub>2</sub> 1243.48

Isoquinolinium, 2,2'-[1,5-pentanediy]bis[oxy(3-oxo-3,1-propanediyl)]bis[1-[(3,4-dimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-dibenzene]sulfonate;  
2-(2-Carboxyethyl)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-phenyl-1-yl veratrylisoquinolinium benzenesulfonate, pentamethylene ester [64228-81-5].

### DEFINITION

Atracurium Besylate contains NLT 96.0% and NMT 102.0% of C<sub>65</sub>H<sub>82</sub>N<sub>2</sub>O<sub>18</sub>S<sub>2</sub>, calculated on the anhydrous basis. It contains NLT 5.0% and NMT 6.5% of the *trans-trans* isomer, NLT 34.5% and NMT 38.5% of the *cis-trans* isomer, and NLT 55.0% and NMT 60.0% of the *cis-cis* isomer.

### IDENTIFICATION

• **A. INFRARED ABSORPTION (197K)**

• **B.** The retention times of the three main isomeric peaks of the Sample solution correspond to those of the Standard solution, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Buffer:** 10.2 g of monobasic potassium phosphate in a 1000-mL volumetric flask. Dissolve in 950 mL of water. While stirring, adjust with phosphoric acid to a pH of 3.1, and dilute with water to volume.

**Solution A:** Acetonitrile, methanol, and Buffer (20:5:75)

**Solution B:** Acetonitrile, methanol, and Buffer (20:30:50)

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
15	40	60
25	40	60
30	0	100
45	0	100
50	80	20

**Standard solution:** 1 mg/mL of USP Atracurium Besylate RS in Solution A

**Sample solution:** 1 mg/mL of Atracurium Besylate in Solution A

#### Chromatographic system

(See Chromatography, (621) System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-μm base-deactivated packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20 μL

#### System suitability

**Sample:** Standard solution

[NOTE—Refer to Table 2 for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks

**Relative standard deviation:** NMT 2.0%, for the *cis-cis* isomer peak

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of atracurium besylate (C<sub>65</sub>H<sub>82</sub>N<sub>2</sub>O<sub>18</sub>S<sub>2</sub>) in the portion of Atracurium Besylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the Sample solution

*r<sub>S</sub>* = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the Standard solution

*C<sub>S</sub>* = concentration of USP Atracurium Besylate RS in the Standard solution (mg/mL)

*C<sub>U</sub>* = concentration of Atracurium Besylate in the Sample solution (mg/mL)

**Acceptance criteria:** 96.0%–102.0%, calculated on the anhydrous basis. It contains NLT 5.0% and NMT 6.5%



of the *trans-trans* isomer, NLT 34.5% and NMT 38.5% of the *cis-trans* isomer, and NLT 55.0% and NMT 60.0% of the *cis-cis* isomer.

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.2%

**Delete the following:**

- **HEAVY METALS, Method II** (231): 20 ppm (Official 1-Jan-2018)

**• ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, Chromatographic system, and Sample solution: Proceed as directed in the Assay.

Standard solution: 0.01 mg/mL of USP Atracurium Besylate RS in Solution A

System suitability solution: 1 mg/mL of USP Atracurium Besylate RS in Solution A

**System suitability**

Sample: System suitability solution

**Suitability requirements**

Resolution: NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks

**Analysis**

Samples: Standard solution and Sample solution

Record the chromatograms, and measure all of the peak responses, except the three main isomeric peaks. Calculate the percentage of each impurity in the portion of Atracurium Besylate taken:

$$\text{Result} = (r_u/r_T) \times (C_S/C_U) \times (1/F) \times 100$$

$r_u$  = peak response for each impurity from the Sample solution

$r_T$  = sum of peak responses due to the atracurium *cis-cis*, *trans-trans*, and *cis-trans* isomers from the Standard solution

$C_S$  = concentration of USP Atracurium Besylate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Atracurium Besylate in the Sample solution (mg/mL)

$F$  = relative response factor (see Table 2)

Acceptance criteria: See Table 2. [NOTE—Disregard any peak less than 0.05%.]

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity E <sup>a</sup>	0.2	1.0	1.5
Impurity F <sup>b</sup>	0.25	1.0	1.0
Impurity G (laudanosine) <sup>c</sup>	0.3	2.0	1.0
Impurity D	0.45 <sup>d</sup> and 0.5 <sup>e</sup>	1.0	1.5 <sup>p</sup>
Atracurium <i>trans-trans</i> isomer	0.8	—	—
Atracurium <i>cis-trans</i> isomer	0.9	—	—
Atracurium <i>cis-cis</i> isomer	1.0	—	—
Impurity A	1.04 <sup>f</sup> and 1.08 <sup>g</sup>	1.0	1.5 <sup>p</sup>
Impurity I	1.07 <sup>h</sup> and 1.12 <sup>k</sup>	1.0	1.0 <sup>p</sup>
Impurity H	1.07 <sup>h</sup> and 1.12 <sup>i</sup>	1.0	1.0 <sup>p</sup>
Impurity K <sup>i</sup>	1.09 and 1.12	1.0	1.0 <sup>p</sup>
Impurity B <sup>m</sup>	1.15	1.0	0.1
Impurity C	1.2 <sup>n</sup> and 1.3 <sup>o</sup>	1.0	1.0 <sup>p</sup>
Any individual impurity	—	1.0	0.1
Total impurities	—	—	3.5

<sup>a</sup> 3-[1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl]propanoate.

<sup>b</sup> 1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium.

<sup>c</sup> 1-(3,4-Dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline.

<sup>d</sup> *trans* Isomer of 1-(3,4-dimethoxybenzyl)-2-[3-[(5-hydroxypentyl)oxy]-3-oxopropyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.

<sup>e</sup> *cis* Isomer of 1-(3,4-dimethoxybenzyl)-2-[3-[(5-hydroxypentyl)oxy]-3-oxopropyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.

<sup>f</sup> *cis-trans* Isomer of 1-(3,4-dimethoxybenzyl)-2-[13-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]-3,11-dioxo-4,10-dioxatridecyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.

<sup>g</sup> *cis-trans* Isomer of 2,2'-[(3-methylpentane-1,5)-diylbis[oxy(3-oxopropyl)-1,3-diyl]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].

<sup>h</sup> *cis-trans* Isomer of 2,2'-[hexane-1,6-diylbis[oxy(3-oxopropyl)-1,3-diyl]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].

<sup>i</sup> *cis-cis* Isomer of 1-(3,4-dimethoxybenzyl)-2-[13-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]-3,11-dioxo-4,10-dioxatridecyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium.

<sup>j</sup> 2,2'-[(Hexane-1,5)-diylbis[3-oxopropyl-1,3-diyl]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].

<sup>k</sup> *cis-cis* Isomer of 2,2'-[(3-methylpentane-1,5)-diylbis[oxy(3-oxopropyl)-1,3-diyl]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].

<sup>l</sup> *cis-cis* Isomer of 2,2'-[hexane-1,6-diylbis[oxy(3-oxopropyl)-1,3-diyl]]bis[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium].

<sup>m</sup> Pentane-1,5-diyl bis[3-[1-(3,4-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl]propanoate].

<sup>n</sup> *trans* Isomer of 1-(3,4-dimethoxybenzyl)-2-(3,11-dioxo-4,10-dioxatridec-12-enyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium benzenesulfonate.

<sup>o</sup> *cis* Isomer of 1-(3,4-dimethoxybenzyl)-2-(3,11-dioxo-4,10-dioxatridec-12-enyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium benzenesulfonate.

<sup>p</sup> Impurity consists of two isomers that are separated under these conditions; integrate both peaks for the impurity calculations.

**• LIMIT OF IMPURITY J (Methyl Benzenesulfonate)**

Buffer, Solution A, and Solution B: Prepare as directed in the Assay.

Standard stock solution: 0.2 mg/mL of Impurity J (methyl benzenesulfonate) in acetonitrile

Standard solution: 1 µg/mL of Impurity J (methyl benzenesulfonate) in Solution A from Standard stock solution

Sample solution: 10 mg/mL of Atracurium Besylate in Solution A



Mobile phase: See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
15	75	25
25	75	25
30	55	45
38	0	100
45	0	100

**Chromatographic system**

Mode: LC

Detector: UV 217 nm

Column: 4.6-mm × 25-cm; 5-μm base-deactivated packing L1

Flow rate: 1 mL/min

Injection size: 100 μL

**Analysis**

Samples: Standard solution and Sample solution

Measure the responses for the Impurity J (methyl benzenesulfonate) peaks.

Acceptance criteria: NMT 0.01%, the peak response of the Sample solution being NMT that of the Standard solution

**SPECIFIC TESTS**

- **WATER DETERMINATION**, Method I (921): NMT 5.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers, in a cold place. [NOTE—Atracurium Besylate is unstable at room temperature.]
- **USP REFERENCE STANDARDS (11)**  
USP Atracurium Besylate RS

**Atracurium Besylate Injection****DEFINITION**

Atracurium Besylate Injection is a sterile solution containing NLT 90.0% and NMT 115.0% of the labeled amount of atracurium besylate ( $C_{65}H_{82}N_2O_{18}S_2$ ). It contains an amount of the *trans-trans* isomer equivalent to NLT 5.0% and NMT 6.5% of the labeled amount of atracurium besylate, an amount of the *cis-trans* isomer equivalent to NLT 34.5% and NMT 38.5% of the labeled amount of atracurium besylate, and an amount of the *cis-cis* isomer equivalent to NLT 55.0% and NMT 60.0% of the labeled amount of atracurium besylate.

[NOTE—The Injection is unstable at room temperature. Store all samples in the refrigerator. Analyze all preparations as soon as possible, or use a refrigerated injector.]

**IDENTIFICATION**

- **A**. The retention times of the peaks of the three atracurium besylate isomers from the Sample solution correspond to those from the Standard solution, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

Buffer: 10.2 g of monobasic potassium phosphate in a 1000-mL volumetric flask. Dissolve in 950 mL of water. While stirring, adjust with phosphoric acid to a pH of 3.1, and dilute with water to volume.

Solution A: Acetonitrile, methanol, and Buffer (20:5:75)

Solution B: Acetonitrile, methanol, and Buffer (20:30:50)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
5	80	20
15	40	60
25	40	60
30	0	100
45	0	100
50	80	20

Standard solution: 1 mg/mL of USP Atracurium Besylate RS in Solution A

Sample solution: Nominally equivalent to 1 mg/mL of atracurium besylate from Injection in Solution A

**Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm base-deactivated packing L1

Flow rate: 1 mL/min

Injection size: 20 μL

**System suitability**

Sample: Standard solution

[NOTE—Refer to Table 2 under Organic Impurities for relative retention times.]

**Suitability requirements**

Resolution: NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks

Relative standard deviation: NMT 2.0%, for the *cis-cis* isomer peak**Analysis**

Samples: Standard solution and Sample solution

Measure the responses for the three atracurium besylate isomer peaks.

Calculate the percentage of the labeled amount of atracurium besylate ( $C_{65}H_{82}N_2O_{18}S_2$ ) in each mL of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the Sample solution

$r_S$  = sum of the peak responses for the *trans-trans* isomer, the *trans-cis* isomer, and the *cis-cis* isomer from the Standard solution

$C_S$  = concentration of USP Atracurium Besylate RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of atracurium besylate in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–115.0% of the labeled amount of atracurium besylate ( $C_{65}H_{82}N_2O_{18}S_2$ ). It contains NLT 5.0% and NMT 6.5% of the *trans-trans* isomer, NLT 34.5% and NMT 38.5% of the *cis-trans* isomer, and NLT 55.0% and NMT 60.0% of the *cis-cis* isomer.

**IMPURITIES**• **ORGANIC IMPURITIES**

Buffer, Solution A, Solution B, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard stock solution: 1 mg/mL of USP Atracurium Besylate RS in Solution A

Standard solution: 0.02 mg/mL of USP Atracurium Besylate RS in Solution A, from Standard stock solution



**System suitability****Sample:** *Standard solution***Suitability requirements**

**Resolution:** NLT 1.5 between the atracurium *trans-trans* isomer and the *cis-trans* isomer peaks; NLT 1.5 between the atracurium *cis-trans* isomer and the *cis-cis* isomer peaks

**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of each impurity in the portion of *Sample solution* taken:

$$\text{Result} = (r_U/r_T) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_T$  = sum of all the peak responses from the *Standard solution*

$C_S$  = concentration of USP Atracurium Besylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of atracurium besylate in the *Sample solution* (mg/mL)

$F$  = relative response factor (see Table 2)

**Acceptance criteria:** See Table 2.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzenesulfonic acid <sup>a</sup>	0.08	—	—
Acidic compound	0.22	1.0	6.0
Impurity G (laudanosine)	0.29	2.0	3.0
<i>cis-</i> and <i>trans-</i> isomers of the hydroxy compound	0.44 <sup>b</sup> and 0.50 <sup>c</sup>	1.0	6.0 <sup>d</sup>
Atracurium <i>trans-trans</i> isomer	0.8	—	—
Atracurium <i>cis-trans</i> isomer	0.9	—	—
Atracurium <i>cis-cis</i> isomer	1.0	—	—
<i>cis-</i> and <i>trans-</i> isomers of the monoacrylate	1.28 <sup>d</sup> and 1.33 <sup>e</sup>	1.0	3.0 <sup>f</sup>
Any individual unspecified degradation product	—	1.0	0.1
Total impurities	—	—	15.0

<sup>a</sup> For identification purposes only.

<sup>b</sup> *trans* isomer of the hydroxy compound.

<sup>c</sup> *cis* isomer of the hydroxy compound.

<sup>d</sup> *trans* isomer of the monoacrylate.

<sup>e</sup> *cis* isomer of the monoacrylate.

<sup>f</sup> Impurity consists of two isomers that are separated under these conditions; integrate both peaks for the impurity calculations.

**SPECIFIC TESTS**

• **pH (791):** 3.00–3.65

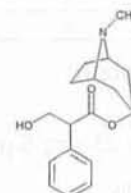
• **STERILITY TESTS (71):** It meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined, Membrane Filtration*.

• **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 5.56 USP Endotoxin Units/mg of atracurium besylate.

• **INJECTIONS AND IMPLANTED DRUG PRODUCTS (1):** Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, in a refrigerator, and protect from freezing. Protect from light.
- **USP REFERENCE STANDARDS (11)**  
USP Atracurium Besylate RS

**Atropine**

$C_{17}H_{23}NO_3$  289.37  
Benzeneacetic acid,  $\alpha$ -(hydroxymethyl)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, *endo*-(±)-;  
1 $\alpha$ H,5 $\alpha$ H-Tropan-3 $\alpha$ -ol (±)-tropate (ester) [51-55-8].

**DEFINITION**

Atropine contains NLT 99.0% and NMT 100.5% of atropine ( $C_{17}H_{23}NO_3$ ), calculated on the anhydrous basis.

[**CAUTION**—Handle Atropine with exceptional care, because it is highly potent.]

**IDENTIFICATION**• **A.**

**Standard:** 36 mg of USP Atropine Sulfate RS

**Sample:** 30 mg

**Analysis:** Dissolve the *Standard* and *Sample* in individual 60-mL separators with the aid of 5-mL portions of water. To each separator add 1.5 mL of 1 N sodium hydroxide solution and 10 mL of chloroform. Shake for 1 min, allow the layers to separate, and pass the chloroform extracts through separate filters of 2 g of anhydrous granular sodium sulfate supported on pledgets of glass wool. Extract each aqueous layer with two additional 10-mL portions of chloroform, filtering and combining with the respective main extracts. Evaporate the chloroform solutions under reduced pressure to dryness, and dissolve each residue in 10 mL of carbon disulfide.

**Acceptance criteria:** The IR absorption spectrum, determined in a 1-mm cell, of the solution of the *Sample* exhibits maxima only at the same wavelengths as that of the solution of the *Standard*.

• **B.**

**Sample solution:** A solution (1 in 50) in 3 N hydrochloric acid

**Analysis:** Add gold chloride TS to the *Sample solution*.

**Acceptance criteria:** A lusterless precipitate is formed (distinction from hyoscyamine, which, similarly treated, yields a lustrous precipitate).

**ASSAY**• **PROCEDURE**

**Sample:** 400 mg of Atropine

**Analysis:** Dissolve the *Sample* in 50 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS to a green endpoint, using 1 drop of crystal violet TS. Perform a blank determination (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 28.94 mg of atropine ( $C_{17}H_{23}NO_3$ ).

**Acceptance criteria:** 99.0%–100.5% on the anhydrous basis



**IMPURITIES**

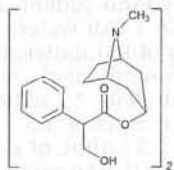
- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **LIMIT OF FOREIGN ALKALOIDS AND OTHER IMPURITIES**  
Standard solution: 24 mg/mL of USP Atropine Sulfate RS in methanol  
Sample solution A: 20 mg/mL of Atropine in methanol  
Sample solution B: 1 mg/mL of Atropine in methanol  
**Chromatographic system**  
(See *Chromatography* (621), *Thin-Layer Chromatography*.)  
Mode: TLC  
Adsorbent: 0.5-mm layer of chromatographic silica gel  
Application volume: See *Analysis*.  
Developing solvent system: Chloroform, acetone, and diethylamine (5:4:1)  
Spray reagent: Potassium iodoplatinate TS  
**Analysis**  
Samples: Standard solution, 5  $\mu$ L; Sample solution A, 25  $\mu$ L; Sample solution B, 1  $\mu$ L  
Apply the Samples to the TLC plate. Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Allow the solvent to evaporate. Locate the spots on the plate by spraying with *Spray reagent*.  
Acceptance criteria: NMT 0.2%; the  $R_f$  value of the principal spot of each *Sample solution* corresponds to that of the *Standard solution*; no secondary spot of *Sample solution A* exhibits intensity equal to or greater than the principal spot of *Sample solution B*.
- **READILY CARBONIZABLE SUBSTANCES TEST** (271)  
Sample solution: 200 mg in 5 mL of 2 N sulfuric acid  
Acceptance criteria: The solution has no more color than *Matching Fluid A*, and the solution is colored no more than light yellow upon the addition of 0.2 mL of nitric acid.

**SPECIFIC TESTS**

- **OPTICAL ROTATION**, *Angular Rotation* (781)  
Sample solution: 1 g, previously dried at 105° for 1 h, in sufficient 50% alcohol (w/w) to obtain a volume of 20 mL at 25° (using a 200-mm tube)  
Acceptance criteria:  $-0.70^\circ$  to  $+0.05^\circ$  (limit of hyoscyamine)
- **MELTING RANGE OR TEMPERATURE** (741): 114°–118°
- **WATER DETERMINATION**, *Method I* (921): NMT 0.2%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Atropine Sulfate RS

**Atropine Sulfate**
 $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ 

Anhydrous

694.83

676.83

Benzeneacetic acid,  $\alpha$ -(hydroxymethyl)-, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester, *endo*-( $\pm$ )-, sulfate (2:1) (salt), monohydrate;

1 $\alpha$ H,5 $\alpha$ H-Tropan-3- $\alpha$ -ol ( $\pm$ )-tropate (ester), sulfate (2:1) (salt) monohydrate [5908-99-6].  
Anhydrous [55-48-1].

**DEFINITION**

Atropine Sulfate contains NLT 98.0% and NMT 102.0% of atropine sulfate ( $C_{17}H_{23}NO_3 \cdot H_2SO_4$ ), calculated on the anhydrous basis.

[**CAUTION**—Handle Atropine Sulfate with exceptional care, because it is highly potent.]

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL**, *Sulfate* (191)  
Sample solution: 50 mg/mL  
Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak in the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in the *Assay*.

**ASSAY**

- **PROCEDURE**  
Buffer: 1.8 g/L of monobasic potassium phosphate and 2.5 g/L of sodium 1-pentanesulfonate, adjusted with phosphoric acid to a pH of 2.5  
Diluent: Acetonitrile and *Buffer* (20:80)  
Solution A: Acetonitrile and *Buffer* (5:95)  
Solution B: Acetonitrile and *Buffer* (80:20)  
Mobile phase: See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	92	8
11	79	21
15	46	54
15.1	92	8
20	92	8

[NOTE—The gradient was established on an HPLC system with a dwell volume of approximately 0.8 mL.]

**System suitability solution:** 1  $\mu$ g/mL of USP Hyoscyamine Related Compound A RS and 0.5 mg/mL of USP Atropine Sulfate RS in *Diluent*

**Standard solution:** 0.5 mg/mL of USP Atropine Sulfate RS in *Diluent*

**Sensitivity solution:** 0.25  $\mu$ g/mL of USP Atropine Sulfate RS in *Diluent*

**Sample solution:** 0.5 mg/mL of Atropine Sulfate in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm  $\times$  15-cm; 3- $\mu$ m packing L1

Column temperature: 50°

Flow rate: 2 mL/min

Injection volume: 5  $\mu$ L

**System suitability**

Samples: *System suitability solution* and *Sensitivity solution*

**Suitability requirements**

[NOTE—See *Table 2* for the relative retention times.]

**Resolution:** NLT 1.4 between hyoscyamine related compound A and atropine, *System suitability solution*

**Tailing factor:** 0.8–1.8 for atropine, *System suitability solution*

**Signal-to-noise ratio:** NLT 10 for atropine, *Sensitivity solution*

**Relative standard deviation:** NMT 1.0% for atropine, *System suitability solution*



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of atropine sulfate ( $C_{17}H_{23}NO_3 \cdot H_2SO_4$ ) in the portion of Atropine Sulfate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of atropine from the *Sample solution*  
 $r_s$  = peak response of atropine from the *Standard solution*  
 $C_s$  = concentration of USP Atropine Sulfate RS in the *Standard solution*  
 $C_u$  = concentration of Atropine Sulfate in the *Sample solution*

**Acceptance criteria:** NLT 98.0% and NMT 102.0% on the anhydrous basis

**IMPURITIES**

- RESIDUE ON IGNITION (281):** NMT 0.2%

- ORGANIC IMPURITIES**

**Buffer, Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Sensitivity solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Atropine Sulfate taken:

$$\text{Result} = (r_u/r_r) \times (1/F) \times 100$$

- $r_u$  = peak response of each impurity from the *Sample solution*  
 $r_r$  = sum of all the peak responses from the *Sample solution*  
 $F$  = relative response factor for each impurity (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tropic acid <sup>a</sup>	0.56	2.1	0.2
7-Hydroxyhyoscyamine <sup>b</sup>	0.66	1.0	0.2
Scopolamine <sup>c</sup>	0.72	1.0	0.2
6-Hydroxyhyoscyamine <sup>d</sup>	0.75	1.0	0.2
Hyoscyamine related compound A	0.97	1.2	0.3
Atropine	1.0	1.0	—
Littorine <sup>e</sup>	1.13	1.2	0.2
Apoatropine <sup>f</sup>	1.60	2.0	0.2

<sup>a</sup> 3-Hydroxy-2-phenylpropanoic acid; also known as (2*RS*)-3-hydroxy-2-phenylpropanoic acid.

<sup>b</sup> (1*S*,3*R*,5*S*)-6-Hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (5*S*)-3-hydroxy-2-phenylpropanoate; also known as (1*S*,3*R*,5*S*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate.

<sup>c</sup> (5*S*)-(1*R*,2*R*,4*S*,5*S*,7*S*)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.0<sup>2,4</sup>]nonan-7-yl 3-hydroxy-2-phenylpropanoate; also known as (5*S*)-(1*R*,2*R*,4*S*,5*S*,7*S*)-9-methyl-3-oxa-9-azatricyclo[3.3.1.0<sup>2,4</sup>]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate.

<sup>d</sup> (1*R*,3*S*,5*R*)-6-Hydroxy-8-methyl-8-azabicyclo[3.2.1]octan-3-yl (5*S*)-3-hydroxy-2-phenylpropanoate; also known as (1*R*,3*S*,5*R*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate.

<sup>e</sup> (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl 2-hydroxy-3-phenylpropanoate; also known as (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-2-hydroxy-3-phenylpropanoate.

<sup>f</sup> (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl 2-phenylacrylate; also known as (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-phenylpropanoate.

**Table 2 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual, unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.5

<sup>a</sup> 3-Hydroxy-2-phenylpropanoic acid; also known as (2*RS*)-3-hydroxy-2-phenylpropanoic acid.

<sup>b</sup> (1*S*,3*R*,5*S*)-6-Hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (5*S*)-3-hydroxy-2-phenylpropanoate; also known as (1*S*,3*R*,5*S*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate.

<sup>c</sup> (5*S*)-(1*R*,2*R*,4*S*,5*S*,7*S*)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.0<sup>2,4</sup>]nonan-7-yl 3-hydroxy-2-phenylpropanoate; also known as (5*S*)-(1*R*,2*R*,4*S*,5*S*,7*S*)-9-methyl-3-oxa-9-azatricyclo[3.3.1.0<sup>2,4</sup>]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate.

<sup>d</sup> (1*R*,3*S*,5*R*)-6-Hydroxy-8-methyl-8-azabicyclo[3.2.1]octan-3-yl (5*S*)-3-hydroxy-2-phenylpropanoate; also known as (1*R*,3*S*,5*R*,6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate.

<sup>e</sup> (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl 2-hydroxy-3-phenylpropanoate; also known as (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*RS*)-2-hydroxy-3-phenylpropanoate.

<sup>f</sup> (1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]octan-3-yl 2-phenylacrylate; also known as (1*R*,3*r*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl 2-phenylpropanoate.

**SPECIFIC TESTS**

- OPTICAL ROTATION, Specific Rotation (781)**

**Sample solution:** 0.1 g/mL of Atropine Sulfate in water

**Acceptance criteria:** Between  $-0.50^\circ$  and  $+0.05^\circ$

- WATER DETERMINATION, Method I (921):** NMT 4.0%

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- USP REFERENCE STANDARDS (11)**

USP Atropine Sulfate RS

USP Hyoscyamine Related Compound A RS

Norhyoscyamine sulfate; (1*R*,3*r*,5*S*)-8-Azabicyclo[3.2.1]octan-3-yl (5*S*)-3-hydroxy-2-phenylpropanoate sulfate

(2:1).

( $C_{16}H_{21}NO_3$ )<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> 648.77

**Atropine Sulfate Injection****DEFINITION**

Atropine Sulfate Injection is a sterile solution of Atropine Sulfate in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of atropine sulfate monohydrate [ $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O$ ].

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- PROCEDURE**

**Buffer:** Dissolve 4.1 g of anhydrous sodium acetate and 2.9 mL of glacial acetic acid in 1 L of water.

**Mobile phase:** Transfer 5.1 g of tetrabutylammonium hydrogen sulfate to a 1-L volumetric flask. Add 50 mL of acetonitrile, and dilute with *Buffer* to volume. Adjust with 5 N sodium hydroxide to a pH of 5.5.

**System suitability solution:** 0.5 µg/mL of *p*-hydroxybenzoic acid and 64 µg/mL of USP Atropine Sulfate RS in water

**Standard solution:** 80 µg/mL of USP Atropine Sulfate RS

**Sample solution:** Nominally equivalent to 80 µg/mL of atropine sulfate in water, from a volume of the *Injection* containing an amount equivalent to 2 mg of atropine sulfate



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 30-cm × 3.9-mm; packing L1

Flow rate: 2 mL/min

Injection volume: 100 µL

**System suitability**Samples: *System suitability solution* and *Standard solution*[NOTE—The relative retention times of atropine and *p*-hydroxybenzoic acid are 1.0 and 1.6, respectively.]**Suitability requirements**Resolution: NLT 2.2 between *p*-hydroxybenzoic acid and atropine, *System suitability solution*Relative standard deviation: NMT 1.5%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of atropine sulfate monohydrate [(C<sub>17</sub>H<sub>23</sub>NO<sub>3</sub>)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> · H<sub>2</sub>O] in the portion of the injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution**r<sub>S</sub>* = peak response from the *Standard solution**C<sub>S</sub>* = concentration of USP Atropine Sulfate RS in the *Standard solution* (mg/mL)*C<sub>U</sub>* = nominal concentration of atropine sulfate in the *Sample solution* (mg/mL)*M<sub>r1</sub>* = molecular weight of atropine sulfate monohydrate, 694.85*M<sub>r2</sub>* = molecular weight of anhydrous atropine sulfate, 676.83

Acceptance criteria: 93.0%–107.0%

**SPECIFIC TESTS**• **PH (791):** 3.0–6.5• **BACTERIAL ENDOTOXINS TEST (85):** NMT 55.6 USP Endotoxin Units/mg of atropine sulfate• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store at controlled room temperature.• **USP REFERENCE STANDARDS (11)**

USP Atropine Sulfate RS

USP Endotoxin RS

**Atropine Sulfate Ophthalmic Ointment****DEFINITION**Atropine Sulfate Ophthalmic Ointment is Atropine Sulfate in a suitable ophthalmic ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of atropine sulfate monohydrate [(C<sub>17</sub>H<sub>23</sub>NO<sub>3</sub>)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> · H<sub>2</sub>O]. It is sterile.**IDENTIFICATION**• **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181)**

Standard solution: Proceed as directed in the chapter.

Sample solution: Transfer a portion of Ophthalmic Ointment, equivalent to 50 mg of atropine sulfate, to a suitable separator, and dissolve in 25 mL of ether. Add 25 mL of 0.01 N hydrochloric acid, shake vigorously, allow the layers to separate, and discard the organic phase. Heat the aqueous phase gently on a steam bath while passing nitrogen through the solution to expel any residual ether.

Instrumental conditions and Analysis: Proceed as directed in the chapter.

Acceptance criteria: Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL, Sulfate (191)**

Sample solution: Transfer 5 g of Ophthalmic Ointment to a separator, dissolve in 50 mL of ether, and extract with 20 mL of water.

Acceptance criteria: Meets the requirements

**ASSAY**• **PROCEDURE**

Buffer: 34.8 g of dibasic potassium phosphate in 900 mL of water. Adjust to a pH of 9.0 by the addition of 3 M hydrochloric acid or 1 M sodium hydroxide, as necessary.

Internal standard solution: 0.5 mg/mL of homatropine hydrobromide in water. Prepare fresh daily.

Standard stock solution: 0.1 mg/mL of USP Atropine Sulfate RS in water

Standard solution: 0.5 mg/mL of atropine sulfate prepared as follows. Pipet 10 mL of *Standard stock solution* into a separator, add 2.0 mL of *Internal standard solution* and 5.0 mL of *Buffer*, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate to near-dryness under a stream of nitrogen. Dissolve the residue in 2.0 mL of methylene chloride. Prepare fresh daily.Sample solution: Nominally 0.5 mg/mL of atropine sulfate prepared as follows. Transfer Ophthalmic Ointment, equivalent to 10 mg of atropine sulfate, to a separator containing 50 mL of ether. Shake to dissolve, extract with three 25-mL portions of 0.1 M sulfuric acid, collect the acid extracts in a 100-mL volumetric flask, and dilute with 0.1 M sulfuric acid to volume. Pipet 10 mL of this solution and treat as follows. Add 2.0 mL of *Internal standard solution* and 5.0 mL of *Buffer*, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate to near-dryness under a stream of nitrogen. Dissolve the residue in 2.0 mL of methylene chloride.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m glass; packed with a 3% phase G3 on support S1AB

**Temperatures**

Column: 225°

Injection port: 250°

Detector: 250°

Flow rate: 25 mL/min

Carrier gas: Nitrogen

Injection volume: 1 µL

**System suitability**Sample: *Standard solution***Suitability requirements**

Resolution: NLT 4.0

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of atropine sulfate monohydrate [(C<sub>17</sub>H<sub>23</sub>NO<sub>3</sub>)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> · H<sub>2</sub>O] in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$



- $R_U$  = peak area ratio of atropine to homatropine from the *Sample solution*  
 $R_S$  = peak area ratio of atropine to homatropine from the *Standard solution*  
 $C_S$  = concentration of USP Atropine Sulfate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of atropine sulfate monohydrate, 694.85  
 $M_{r2}$  = molecular weight of anhydrous atropine sulfate, 676.83  
 Acceptance criteria: 90.0%–110.0%

**SPECIFIC TESTS**

- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS**: It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** (11)  
USP Atropine Sulfate RS

**Atropine Sulfate Ophthalmic Solution****DEFINITION**

Atropine Sulfate Ophthalmic Solution is a sterile, aqueous solution of Atropine Sulfate. It contains NLT 93.0% and NMT 107.0% of the labeled amount of atropine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ . It may contain suitable stabilizers and antimicrobial agents.

**IDENTIFICATION**• **A. INFRARED ABSORPTION** (197M)

**Standard**: 36 mg USP Atropine Sulfate RS

**Sample**: Ophthalmic Solution, equivalent to 30 mg, evaporated to dryness

**Analysis**: Dissolve the *Sample* and *Standard* in individual 60-mL separators with the aid of 5-mL portions of water. To each separator add 1.5 mL of 1 N sodium hydroxide solution and 10 mL of chloroform. Shake for 1 min, allow the layers to separate, and pass the chloroform extracts through separate filters of 2 g of anhydrous granular sodium sulfate supported on pledgets of glass wool. Extract each aqueous layer with two additional 10-mL portions of chloroform, filtering and combining with the respective main extracts. Evaporate the chloroform solutions under reduced pressure to dryness, and dissolve each residue in 10 mL of carbon disulfide.

**Acceptance criteria**: The IR absorption spectrum, determined in a 1-mm cell, of the solution of the *Sample* exhibits maxima only at the same wavelengths as that of the solution of the *Standard*.

• **B. IDENTIFICATION TESTS—GENERAL, Sulfate** (191)

**Sample solution**: Evaporate to dryness a quantity of Ophthalmic Solution. Prepare a solution from the residue that contains the equivalent of 50 mg of atropine sulfate/mL.

**Acceptance criteria**: Meets the requirements

**ASSAY**• **PROCEDURE**

**Buffer**: 34.8 g of dibasic potassium phosphate in 900 mL of water. Adjust to a pH of 9.0 by the addition of 3 M hydrochloric acid or 1 M sodium hydroxide.

**Internal standard solution**: 0.5 mg/mL of homatropine hydrobromide in water. [NOTE—Prepare fresh daily.]

**Standard stock solution**: 0.1 mg/mL of USP Atropine Sulfate RS in water

**Standard solution**: 0.5 mg/mL of atropine sulfate prepared as follows. Pipet 10 mL of *Standard stock solution* into a separator, add 2.0 mL of *Internal standard solution* and 5.0 mL of *Buffer*, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under a stream of nitrogen to near-dryness. Dissolve the residue in 2.0 mL of methylene chloride. [NOTE—Prepare fresh daily.]

**Sample solution**: Nominally 0.5 mg/mL of atropine sulfate prepared as follows. Ophthalmic Solution, equivalent to 10 mg of atropine sulfate, in a 100-mL volumetric flask. Dilute with water to volume. Pipet 10 mL of this solution and treat as follows. Add 2.0 mL of *Internal standard solution* and 5.0 mL of *Buffer*, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under a stream of nitrogen to near-dryness. Dissolve the residue in 2.0 mL of methylene chloride.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode**: GC

**Detector**: Flame ionization

**Column**: 2-mm  $\times$  1.8-m glass; packed with a 3% phase G3 on support S1AB

**Temperatures**

**Column**: 225°

**Injection port**: 250°

**Detector**: 250°

**Flow rate**: 25 mL/min

**Carrier gas**: Nitrogen

**Injection volume**: 1  $\mu$ L

**System suitability**

**Sample**: *Standard solution*

**Suitability requirements**

**Resolution**: NLT 4.0

**Tailing factor**: NMT 2.0

**Relative standard deviation**: NMT 2.0%

**Analysis**

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of atropine sulfate

$[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$  in each mL of Ophthalmic Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak area ratio of atropine to homatropine from the *Sample solution*

$R_S$  = peak area ratio of atropine to homatropine from the *Standard solution*

$C_S$  = concentration of USP Atropine Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of atropine sulfate monohydrate, 694.85

$M_{r2}$  = molecular weight of anhydrous atropine sulfate, 676.83



Acceptance criteria: 93.0%–107.0%

#### SPECIFIC TESTS

- **pH** (791): 3.5–6.0
- **STERILITY TESTS** (71): Meets the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Atropine Sulfate RS

## Atropine Sulfate Tablets

#### DEFINITION

Atropine Sulfate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of atropine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$ .

#### IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** (181)  
Sample: A quantity of Tablets, equivalent to 5 mg of atropine sulfate  
Analysis: Triturate with 10 mL of water for a few min, and filter into a small separator. Render the solution alkaline with 6 N ammonium hydroxide, and extract with 50 mL of chloroform. Filter the chloroform layer, and evaporate to dryness.  
Acceptance criteria: The residue meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Sulfate** (191): A filtered solution of Tablets meets the requirements of the tests.

#### ASSAY

- **PROCEDURE**  
Buffer: 34.8 g of dibasic potassium phosphate in 900 mL of water. Adjust to a pH of 9.0 by the addition of 3 M hydrochloric acid or 1 M sodium hydroxide, as necessary.  
Internal standard solution: 0.5 mg/mL of homatropine hydrobromide in water. [NOTE—Prepare fresh daily.]  
Standard solution: 0.1 mg/mL of USP Atropine Sulfate RS in water. Pipet 10 mL of this solution into a separator, add 2.0 mL of Internal standard solution and 5.0 mL of Buffer, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under a stream of nitrogen to near-dryness. Dissolve the residue in 2.0 mL of methylene chloride. [NOTE—Prepare fresh daily.]  
Sample solution: Transfer an equivalent to 1 mg of atropine sulfate, from NLT 20 finely powdered Tablets, to a separator. Add 2.0 mL of Internal standard solution and 5.0 mL of Buffer, and adjust the solution in the separator with 1 M sodium hydroxide to a pH of 9.0. Extract with two 10-mL portions of methylene chloride, filter the methylene chloride extracts through 1 g of anhydrous sodium sulfate supported by a small cotton plug in a funnel into a 50-mL beaker, and evaporate under a stream of nitrogen to near-dryness. Dissolve the residue in 2.0 mL of methylene chloride.  
**Chromatographic system**  
(See Chromatography (621), System Suitability.)  
Mode: GC  
Detector: Flame ionization  
Column: 2-mm  $\times$  1.8-m glass; packed with a 3% phase G3 on support S1AB

#### Temperatures

Column: 225°  
Injection port: 250°  
Detector: 250°  
Flow rate: 25 mL/min  
Carrier gas: Nitrogen  
Injection volume: 1  $\mu$ L  
System suitability  
Sample: Standard solution  
Suitability requirements  
Resolution: NLT 4.0  
Tailing factor: NMT 2.0  
Relative standard deviation: NMT 2.0%

#### Analysis

Samples: Standard solution and Sample solution  
Calculate the percentage of the labeled amount of atropine sulfate  $[(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O]$  in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $R_U$  = peak area ratio of atropine to homatropine from the Sample solution  
 $R_S$  = peak area ratio of atropine to homatropine from the Standard solution  
 $C_S$  = concentration of USP Atropine Sulfate RS in the Standard solution (mg/mL)  
 $C_U$  = nominal concentration of atropine sulfate in the Sample solution (mg/mL)  
 $M_{r1}$  = molecular weight of atropine sulfate monohydrate, 694.85  
 $M_{r2}$  = molecular weight of anhydrous atropine sulfate, 676.83  
Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

- **DISINTEGRATION** (701)  
Time: 15 min  
Acceptance criteria: Meet the requirements
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP Atropine Sulfate RS

## Activated Attapulgate

» Activated Attapulgate is a highly heat-treated, processed, native magnesium aluminum silicate.

**Packaging and storage**—Preserve in well-closed containers.

**Identification**—Activated Attapulgate responds to the Identification test for Colloidal Activated Attapulgate, the characteristic peak, however, being much less intense.

**Loss on drying** (731)—Dry it at 105° to constant weight: it loses not more than 4.0% of its weight.

**Loss on ignition** (733)—When ignited at 1000° for 1 hour, it loses between 4.0% and 12.0% of its weight.

**Volatile matter**—When ignited at 600° for 1 hour, it loses between 3.0% and 7.5% of its weight on the dried basis.

**Powder fineness**—Proceed as directed in the test for Powder fineness under Colloidal Activated Attapulgate. The dry weight of the residue is not more than 0.10% of the weight of the specimen taken.

**Acid-soluble matter**—Boil 2.0 g with 100 mL of 0.2 N hydrochloric acid for 5 minutes, and cool. Add water to adjust



the volume to 100 mL, and filter. Evaporate 50 mL of the filtrate so obtained to dryness, and ignite the residue at 600°: not more than 0.25 g is found (25%).

**Other requirements**—It meets the requirements of the tests for *Microbial limits*, *pH*, *Carbonate*, *Arsenic and Lead*, and *Adsorptive capacity* under *Colloidal Activated Attapulgit*.

## Colloidal Activated Attapulgit

» Colloidal Activated Attapulgit is a purified native magnesium aluminum silicate.

**Packaging and storage**—Preserve in well-closed containers.

**Identification**—Add 2 g in small portions to 100 mL of water, with vigorous agitation. Allow to stand for at least 12 hours to ensure complete hydration. Place 2 mL of the resulting mixture on a suitable glass slide, and allow to air-dry at room temperature to produce a uniform film. Place the slide in a vacuum desiccator over a free surface of ethylene glycol. Evacuate the desiccator, and close the stopcock so that the ethylene glycol saturates the desiccator chamber. Allow to stand for 12 hours. Record the X-ray diffraction pattern (see *X-Ray Diffraction* (941)), and calculate the *d* values: several peaks are observed; the characteristic peak corresponds to a *d* value between 10.3 and 10.7 Angstrom units.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the test for absence of *Escherichia coli*.

**pH** (791)—Disperse 1.0 g in 10 mL of carbon dioxide-free water, and mix: the pH of the mixed dispersion so obtained is between 7.0 and 9.5.

**Loss on drying** (731)—Dry it at 105° to constant weight: it loses between 5.0% and 17.0% of its weight.

**Loss on ignition** (733)—When ignited at 1000° for 1 hour, it loses between 17.0% and 27.0% of its weight.

**Volatile matter**—When ignited at 600° for 1 hour, it loses between 7.5% and 12.5% of its weight on the dried basis.

**Powder fineness**—Add 50 g to 450 mL of water containing 5 g of sodium pyrophosphate, and stir for 10 minutes. Pour the resulting dispersion slowly through a No. 325 standard sieve (see *Particle Size Distribution Estimation by Analytical Sieving* (786)), and carefully wash the residue until clean. Dry the residue at 105° to constant weight: the dry weight of the residue so obtained is not more than 0.30% of the weight of the specimen taken.

**Acid-soluble matter**—Boil 2.0 g with 100 mL of 0.2 N hydrochloric acid for 5 minutes, and cool. Add water to adjust the volume to 100 mL, and filter. Evaporate 50 mL of the filtrate so obtained to dryness, and ignite the residue at 600°: not more than 0.15 g is found (15%).

**Carbonate**—Mix 1.0 g with 15 mL of 0.5 N sulfuric acid: no effervescence occurs.

**Arsenic and Lead**—To 5.0 g add 50 mL of 1 N nitric acid, and boil for 30 minutes, adding 1 N nitric acid at times to maintain the volume. Filter into a 100-mL volumetric flask, wash the filter with water, and dilute the combined filtrate and washings with water to volume.

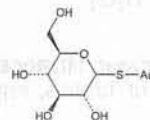
**Arsenic**—Determine the arsenic in the solution by atomic absorption spectrometry (see *Atomic Absorption Spectroscopy* (852)), using a graphite furnace to volatilize the arsenic, as directed by the manufacturer of the instrument used, and measuring the absorbance at 189.0 nm against a standard: not more than 2 ppm is found.

**Lead**—Determine the lead in the solution by atomic absorption spectrometry (see *Atomic Absorption Spectroscopy*

(852)), using a graphite furnace to volatilize the lead, as directed by the manufacturer of the instrument used, and measuring the absorbance at 283.3 nm against a standard: not more than 0.001% is found.

**Adsorptive capacity**—To 10 mL of a 1 in 10 suspension of the specimen in water add 80 mL of methylene blue solution (1 in 1000), and shake. Add 10 mL of barium chloride solution (1 in 50), and shake. Allow to stand for 15 minutes. Transfer 40 mL of the supernatant to a 50-mL centrifuge tube, and centrifuge. To 5 mL of the clear supernatant add 495 mL of water, and mix: the color of the solution so obtained is not deeper than that of a solution containing 1.5 µg of methylene blue per mL.

## Aurothioglucose



$C_6H_{11}AuO_5S$  392.18

Gold, (1-thio-D-glucopyranosato)-.

(1-Thio-D-glucopyranosato)gold [12192-57-3].

» Aurothioglucose contains not less than 95.0 percent and not more than 105.0 percent of  $C_6H_{11}AuO_5S$ , calculated on the dried basis. It is stabilized by the addition of a small amount of Sodium Acetate.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at room temperature.

**USP Reference standards** (11)—

USP Aurothioglucose RS

**Identification**—

**A:** Dissolve a suitable quantity in water to obtain a solution containing 4 mg per mL. Apply 10 µL of this solution and 10 µL of an aqueous Standard solution of USP Aurothioglucose RS containing 4 mg per mL to a suitable thin-layer chromatographic glass microfilament sheet (see *Chromatography* (621)) impregnated with silicic acid and a suitable fluorescing substance. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of *n*-propyl alcohol, water, and ethyl acetate (3:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the sheet from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the *R<sub>f</sub>* value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**B:** To a portion of the filtrate obtained in the Assay add barium chloride TS: a heavy, white precipitate is formed.

**Specific rotation** (781S): between +65° and +75°.

**Test solution:** 10 mg per mL, in water.

**Loss on drying** (731)—Dry it over phosphorus pentoxide for 24 hours: it loses not more than 1.0% of its weight.

**Assay**—Accurately weigh about 1 g of Aurothioglucose, and dissolve in 100 mL of water in a 300-mL Kjeldahl flask. Slowly add 10 mL of nitric acid, and when the reaction has subsided, boil the mixture for 5 minutes. Filter, wash well the separated gold with hot water, dry, and ignite to constant weight. The weight of the gold so obtained, multiplied by 1.991, represents the weight of  $C_6H_{11}AuO_5S$  in the portion of Aurothioglucose taken.



## Aurothioglucose Injectable Suspension

» Aurothioglucose Injectable Suspension is a sterile suspension of Aurothioglucose in a suitable vegetable oil. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_6H_{11}AuO_5S$ . It may contain suitable thickening agents.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass. Protect from light.

### USP Reference standards (11)—

USP Aurothioglucose RS

**Identification**—Transfer a volume of Injectable Suspension, equivalent to about 200 mg of aurothioglucose, to a centrifuge separator containing 20 mL of ethyl acetate and 50 mL of water. Shake the mixture thoroughly, and centrifuge until the liquid phases have been clearly separated. Withdraw the lower, aqueous phase, and filter, discarding the first 10 mL of the filtrate. Collect the filtrate in a glass-stoppered vessel, and proceed as directed in *Identification test A* under *Aurothioglucose*, beginning with "apply 10  $\mu$ L of this solution."

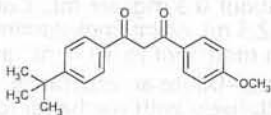
**Bacterial Endotoxins Test** (85)—It contains not more than 7.14 USP Endotoxin Units per mg of aurothioglucose.

**Sterility Tests** (71)—It meets the requirements.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—Transfer with a pipet, calibrated to contain rather than to deliver, an accurately measured volume of Injectable Suspension, equivalent to about 200 mg of aurothioglucose, to a beaker containing 400 mL of acetone. Wash the pipet into the beaker with a small quantity of acetone, mix, allow the solids to settle, and decant the supernatant through a filter. Wash the solids with another 400-mL portion of acetone, and repeat the decantation. Transfer the solids to the filter with the aid of acetone, then transfer the filter and its contents to a short-necked, 300-mL Kjeldahl flask, add 5 mL of water, and proceed as directed in the *Assay* under *Gold Sodium Thiomaleate*, beginning with "add 20 mL of nitric acid." The weight of gold so obtained, multiplied by 1.991, represents the weight of  $C_6H_{11}AuO_5S$  in the portion of Injectable Suspension taken.

## Avobenzone



$C_{20}H_{22}O_3$  310.40  
1,3-Propanedione, 1-[4-(1,1-dimethylethyl)phenyl]-3-(4-methoxyphenyl)-;  
1-(*p*-*tert*-Butylphenyl)-3-(*p*-methoxyphenyl)-1,3-propanedione [70356-09-1].

### DEFINITION

Avobenzone contains NLT 95.0% and NMT 105.0% of avobenzone ( $C_{20}H_{22}O_3$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)

Analytical wavelength: 360 nm

Sample solution: 5  $\mu$ g/mL in alcohol

Acceptance criteria: Absorptivities do not differ by more than 3.0%.

### ASSAY

#### • PROCEDURE

Standard solution: 50 mg/mL of USP Avobenzone RS in acetone

Sample solution: 50 mg/mL of Avobenzone in acetone

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.32-mm  $\times$  25-m fused silica capillary coated with phase G1

Temperatures

Injection port: 200°

Detector: 280°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
200	4	280	—

Carrier gas: Helium

Injection volume: 1  $\mu$ L

Split ratio: 50:1

System suitability

Sample: Standard solution

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of avobenzone ( $C_{20}H_{22}O_3$ ) in the portion of Avobenzone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Avobenzone RS in the Standard solution (mg/mL)

$C_U$  = concentration of Avobenzone in the Sample solution (mg/mL)

Acceptance criteria: 95.0%–105.0% on the dried basis

### IMPURITIES

#### • ORGANIC IMPURITIES

Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Analysis

Sample: Sample solution

Calculate the percentage of each impurity in the portion of Avobenzone taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity from the Sample solution

$r_T$  = sum of all peak responses from the Sample solution



**Acceptance criteria**

Each individual impurity: NMT 3.0%

Total impurities: NMT 4.5%

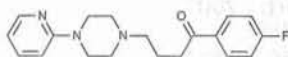
**SPECIFIC TESTS**• **Loss on drying** (731)

Analysis: Dry a sample under vacuum at 70° for 4 h.

Acceptance criteria: NMT 0.5%

**ADDITIONAL REQUIREMENTS**• **Packaging and storage:** Preserve in tight, light-resistant containers.• **USP Reference Standards** (11)

USP Avobenzone RS

**Azaperone** $C_{19}H_{22}FN_3O$  327.40

1-Butanone, 1-(4-fluorophenyl)-4-[4-(2-pyridinyl)-1-piperazinyl]-

4'-Fluoro-4-[4-(2-pyridyl)-1-piperazinyl]butyrophenone [1649-18-9].

» Azaperone contains not less than 98.0 percent and not more than 102.0 percent of  $C_{19}H_{22}FN_3O$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, protected from light. Store at room temperature.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Azaperone RS

**Identification**, *Infrared Absorption* (197K): previously dried.

**Melting range** (741): between 92° and 95°.

**Loss on drying** (731)—Dry it in vacuum at 60° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chromatographic purity**—Dissolve an accurately weighed quantity of USP Azaperone RS in a mixture of acetone and methylene chloride (5:1) to obtain a solution having a concentration of 0.50 mg per mL (*Standard solution A*). Quantitatively dilute a volume of *Standard solution A* with the same solvent mixture to obtain a solution having a concentration of 0.25 mg per mL (*Standard solution B*). Prepare a test solution by dissolving an accurately weighed quantity of Azaperone in a mixture of acetone and methylene chloride (5:1) to obtain a solution containing 50 mg per mL. Separately apply 1  $\mu$ L each of *Standard solution A*, *Standard solution B*, and the test solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.2-mm layer of chromatographic silica gel mixture with chemically bonded amino groups, and allow the spots to dry. Develop the chromatograms in a solvent system consisting of a mixture of cyclohexane, acetone, and methanol (65:30:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, and allow the plate to air-dry. Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots, other than any spot at the origin, observed in the chromatogram of the test solution with those of the principal spots in the chromatograms of *Standard solution A* and *Standard solution B*: the sum of the intensities of the secondary spots obtained from the test solution corresponds to not more than the

intensity of the principal spot in the chromatogram of *Standard solution A* (1.0%).

**Assay**—Dissolve about 120 mg of Azaperone, accurately weighed, in 50 mL of a mixture of methyl ethyl ketone and glacial acetic acid (7:1). Add 3 drops of *p*-naphtholbenzein TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 16.37 mg of  $C_{19}H_{22}FN_3O$ .

**Azaperone Injection**

» Azaperone Injection is a sterile solution of Azaperone in Water for Injection, prepared with the aid of Tartaric Acid. It may contain a suitable preservative and a stabilizing agent. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{19}H_{22}FN_3O$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Azaperone RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for azaperone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

**pH** (791): between 4.0 and 5.6.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—

*Mobile phase*—Prepare a filtered and degassed mixture containing 6 volumes of acetonitrile and 4 volumes of 0.01 M dibasic potassium phosphate, and adjust by the addition of dilute phosphoric acid (1 in 10) to a pH of  $7.8 \pm 0.1$ . Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Prepare a solution of benzo-phenone in methanol containing about 0.5 mg per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Azaperone RS in methanol, and dilute quantitatively with methanol to obtain a solution having a known concentration of about 0.5 mg per mL. Combine 2.5 mL of this solution with 2.5 mL of *Internal standard solution*, dilute quantitatively with methanol to 10.0 mL, and mix.

*Assay preparation*—Dilute an accurately measured volume of Injection quantitatively with methanol to obtain a solution containing about 0.5 mg of azaperone per mL. Combine 2.5 mL of this solution with 2.5 mL of *Internal standard solution*, dilute quantitatively with methanol to 10.0 mL, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with 243-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the azaperone and internal standard peaks is not less than 2.7; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into

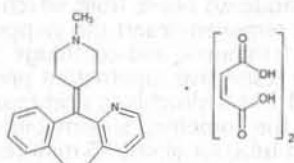


the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of azaperone ( $C_{19}H_{22}FN_3O$ ) in each mL of the Injection taken by the formula:

$$(C)(L/D)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Azaperone RS in the *Standard preparation*; L is the labeled quantity, in mg, of azaperone in each mL of the Injection; D is the concentration, in mg per mL, of azaperone in the *Assay preparation*, based on the volume of Injection taken and the extent of dilution; and  $R_U$  and  $R_S$  are the ratios of the azaperone peak to the benzophenone peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Azatadine Maleate



$C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$  522.55  
5H-Benzo[5,6]cyclohepta[1,2-b]pyridine, 6,11-dihydro-11-(1-methyl-4-piperidylidene)-, (Z)-2-butenedioate (1:2);  
6,11-Dihydro-11-(1-methyl-4-piperidylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine maleate (1:2) [3978-86-7].

### DEFINITION

Azatadine Maleate contains NLT 98.0% and NMT 102.0% of azatadine maleate ( $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B. ULTRAVIOLET ABSORPTION** (197U)  
Medium: 0.25 N hydrochloric acid in methanol  
Sample solution: 40 µg/mL in Medium  
Acceptance criteria: Meets the requirements
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Solution A:** 3.854 g/L of ammonium acetate in water. Adjust with 25% ammonium hydroxide solution to a pH of 7.6 and pass through a suitable filter of 0.2-µm pore size.

**Solution B:** Acetonitrile and methanol (20:80)

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	80	20
7.0	70	30
12.0	60	40
14.0	50	50
16.0	30	70
18.0	30	70
18.1	80	20
20.0	80	20

**Standard solution:** 0.5 mg/mL of USP Azatadine Maleate RS in water

**Sample solution:** 0.5 mg/mL of Azatadine Maleate in water

### Chromatographic system

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 237 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L11

**Column temperature:** 45°

**Flow rate:** 1.2 mL/min

**Injection volume:** 3 µL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 0.73%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of azatadine maleate ( $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$ ) in the portion of Azatadine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of azatadine from the *Sample solution*

$r_S$  = peak response of azatadine from the *Standard solution*

$C_S$  = concentration of USP Azatadine Maleate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Azatadine Maleate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

#### ORGANIC IMPURITIES

**Solution A, Solution B, Mobile phase, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard stock solution:** 1.0 mg/mL of USP Azatadine Maleate RS in water

**Standard solution:** 0.001 mg/mL of USP Azatadine Maleate RS in water from the *Standard stock solution*

**Sample solution:** 1.0 mg/mL of Azatadine Maleate in water

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual unspecified impurity in the portion of Azatadine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any individual unspecified impurity from the *Sample solution*

$r_S$  = peak response of azatadine from the *Standard solution*

$C_S$  = concentration of USP Azatadine Maleate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Azatadine Maleate in the *Sample solution* (mg/mL)

### Acceptance criteria

**Individual impurity:** NMT 0.10%

**Total impurities:** NMT 2.0%. Disregard any impurity peak less than 0.05%.



**SPECIFIC TESTS**• **LOSS ON DRYING** (731)

Analysis: Dry under vacuum at 60° for 3 h.

Acceptance criteria: NMT 1.0%

**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers.• **USP REFERENCE STANDARDS** (11)

USP Azatadine Maleate RS

**Azatadine Maleate Tablets**

» Azatadine Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Azatadine Maleate RS

**Identification**—Transfer 15.0 mL of the *Standard preparation* and 15.0 mL of the *Assay preparation*, respectively, prepared as directed in the *Assay*, to separate 50-mL centrifuge tubes fitted with glass stoppers. To each centrifuge tube add 10.0 mL of 1.0 N sodium hydroxide and 20 mL of solvent hexane, insert the stoppers, rotate the centrifuge tubes for about 15 minutes, and centrifuge. Transfer the solvent hexane extracts (upper phase) from each centrifuge tube to separate 50-mL conical flasks fitted with glass stoppers. Evaporate the solvent hexane extracts on a steam bath under a stream of nitrogen to dryness, pipet 1 mL of solvent hexane into each flask, insert the stoppers, and mix by use of a vortex mixer (or equivalent) until the residues have dissolved. Use these solutions as the *Standard solution* and the *test solution*, respectively. Apply separately 100  $\mu$ L each of the *test solution* and the *Standard solution* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of toluene, isopropyl alcohol, and diethylamine (10:10:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the plate to air-dry. Examine the plate under short-wavelength UV light: the  $R_f$  value and intensity of the principal spot in the chromatogram of the *test solution* correspond to those obtained from the chromatogram of the *Standard solution*.

**Dissolution** (711)—

*Medium:* 0.01 N hydrochloric acid; 500 mL.

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

**Procedure**—Determine the amount of  $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 283 nm on filtered portions of the solution under test, diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Azatadine Maleate RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Azatadine Maleate RS in 0.1 N hydrochloric acid, and dilute quantitatively, and stepwise if necessary,

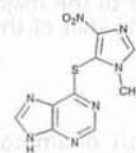
with 0.1 N hydrochloric acid to obtain a solution having a known concentration of about 0.06 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1.5 mg of azatadine maleate, to a 50-mL flask fitted with a glass stopper. Add 25.0 mL of 0.1 N hydrochloric acid, insert the stopper, and shake the mixture by mechanical means for about 30 minutes. Filter the mixture into a suitable glass-stoppered vessel, discarding the first 5 mL of the filtrate.

**Procedure**—Separately transfer 15.0 mL of the *Standard preparation*, 15.0 mL of the *Assay preparation*, and 15.0 mL of 0.1 N hydrochloric acid to provide the reagent blank to three 50-mL centrifuge tubes fitted with glass stoppers. To each centrifuge tube add 10.0 mL of 1.0 N sodium hydroxide and 20 mL of solvent hexane, insert the stoppers, rotate the centrifuge tubes for about 15 minutes, and centrifuge until the supernatants (solvent hexane phase) are clear. With the aid of separate syringes, transfer the supernatants to separate 50-mL centrifuge tubes fitted with glass stoppers. Rinse each syringe with 10 mL of solvent hexane, and add the rinse to the aqueous phase from which the respective supernatant was removed. Insert the stoppers, rotate each tube for about 10 minutes, and centrifuge. Transfer each supernatant to the respective supernatant previously collected. Pipet 15 mL of 0.1 N hydrochloric acid into each centrifuge tube containing the combined supernatants, insert the stoppers, rotate each tube for about 15 minutes, and centrifuge. Remove and discard the supernatants. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 283 nm, with a suitable spectrophotometer zeroed with 0.1 N hydrochloric acid, using the prepared reagent blank. Calculate the quantity, in mg, of  $C_{20}H_{22}N_2 \cdot 2C_4H_4O_4$  in the portion of Tablets taken by the formula:

$$25C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Azatadine Maleate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

**Azathioprine**

$C_9H_7N_7O_2S$  277.26  
1H-Purine, 6-[(1-methyl-4-nitro-1H-imidazol-5-yl)thio]-;  
6-[(1-Methyl-4-nitroimidazol-5-yl)thio]purine [446-86-6].

**DEFINITION**

Azathioprine contains NLT 98.0% and NMT 102.0% of azathioprine ( $C_9H_7N_7O_2S$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Solution A:** 1.6 g/L of sodium 1-heptanesulfonate in water

**Mobile phase:** Methanol and *Solution A* (30:70). Adjust with 1 N hydrochloric acid to a pH of  $3.5 \pm 0.1$ .



**Standard stock solution:** 0.5 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add 25% of the flask volume of methanol and 1% of ammonium hydroxide to the flask. Swirl, and sonicate for 2 min or until dissolved. Dilute with methanol to volume.

**Standard solution:** 0.1 mg/mL of USP Azathioprine RS in water from the *Standard stock solution*

**Sample solution:** 0.1 mg/mL of Azathioprine prepared as follows. Transfer 50 mg of sample to a 100-mL volumetric flask. Add 25 mL of methanol and 1.0 mL of ammonium hydroxide to the flask, swirl, and sonicate for 2 min or until dissolved. Dilute with methanol to volume. Transfer 10.0 mL of this solution into a 50-mL volumetric flask, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; 10-μm packing L1

**Flow rate:** 1.8 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2

**Relative standard deviation:** NMT 0.73%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of azathioprine ( $C_9H_7N_3O_2S$ ) in the portion of Azathioprine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of azathioprine from the *Sample solution*

$r_S$  = peak response of azathioprine from the *Standard solution*

$C_S$  = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Azathioprine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

#### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **ORGANIC IMPURITIES**

**Buffer:** 2.76 g/L of monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 2.5.

**Solution A:** Methanol and *Buffer* (5:95)

**Solution B:** Methanol and *Buffer* (60:40)

**Mobile phase:** See *Table 1*. Return to original conditions, and re-equilibrate the system.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	100	0
15	0	100
20	0	100

**Diluent:** 0.8 g/L of sodium hydroxide in water

**System suitability stock solution A:** 0.2 mg/mL each of USP Azathioprine Related Compound A RS and USP Mercaptopurine RS prepared as follows. Transfer USP Azathioprine Related Compound A RS and USP Mercaptopurine RS to a suitable volumetric flask. Add 35% of the flask volume of *Diluent*, and dilute with *Buffer* to volume.

**System suitability stock solution B:** 0.1 mg/mL each of USP Azathioprine Related Compound G RS and USP

Azathioprine RS prepared as follows. Transfer USP Azathioprine Related Compound G RS and USP Azathioprine RS to a suitable volumetric flask. Add 35% of the flask volume of *Diluent*, and dilute with *Buffer* to volume.

**System suitability solution:** 0.002 mg/mL each of USP Azathioprine Related Compound A RS, USP Mercaptopurine RS, USP Azathioprine Related Compound G RS, and USP Azathioprine RS prepared as follows. Transfer 1 mL of *System suitability stock solution A* and 2 mL of *System suitability stock solution B* to a 100-mL volumetric flask. Add 35 mL of *Diluent*, and dilute with *Buffer* to volume.

**Standard stock solution:** 0.1 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add 35% of the flask volume of *Diluent*, and dilute with *Buffer* to volume.

**Standard solution:** 0.1 μg/mL of USP Azathioprine RS in *Buffer* from *Standard stock solution*

**Sample solution:** 0.1 mg/mL of Azathioprine prepared as follows. Transfer Azathioprine to a suitable volumetric flask. Add 35% of the flask volume with *Diluent*, and dilute with *Buffer* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L11

**Column temperature:** 30°

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 2 between azathioprine related compound A and mercaptopurine; NLT 2 between azathioprine related compound G and azathioprine

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Azathioprine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of azathioprine from the *Standard solution*

$C_S$  = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Azathioprine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 2*. Disregard any impurity peaks less than 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azathioprine related compound A <sup>a</sup>	0.3	0.15
Mercaptopurine <sup>b</sup>	0.4	0.15
Azathioprine related compound G <sup>c</sup>	0.97	0.10
Azathioprine	1.0	—

<sup>a</sup> 1-Methyl-4-nitro-1H-imidazol-5-amine.

<sup>b</sup> 9H-Purine-6-thiol.

<sup>c</sup> 6-[(1-Methyl-4-nitro-1H-imidazol-5-yl)thio]-9H-purin-2-amine.



Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any other unspecified impurity	—	0.10
Total impurities	—	0.5

<sup>a</sup> 1-Methyl-4-nitro-1*H*-imidazol-5-amine.

<sup>b</sup> 9*H*-Purine-6-thiol.

<sup>c</sup> 6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9*H*-purin-2-amine.

## SPECIFIC TESTS

### • LOSS ON DRYING (731)

Analysis: Dry under vacuum at 105° for 5 h.

Acceptance criteria: NMT 1.0%

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

### • USP REFERENCE STANDARDS (11)

USP Azathioprine RS

USP Azathioprine Related Compound A RS

1-Methyl-4-nitro-1*H*-imidazol-5-amine.

C<sub>4</sub>H<sub>6</sub>N<sub>4</sub>O<sub>2</sub> 142.12

USP Azathioprine Related Compound G RS

6-[(1-Methyl-4-nitro-1*H*-imidazol-5-yl)thio]-9*H*-purin-2-amine.

C<sub>9</sub>H<sub>8</sub>N<sub>8</sub>O<sub>2</sub>S 292.28

USP Mercaptopurine RS

## Azathioprine Compounded Oral Suspension

### DEFINITION

Azathioprine Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of azathioprine (C<sub>9</sub>H<sub>7</sub>N<sub>7</sub>O<sub>2</sub>S).

Prepare Azathioprine Compounded Oral Suspension 50 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Azathioprine	5 g
Vehicle: a 1:1 mixture of Vehicle for Oral Solution, (regular or sugar-free), NF and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

If using tablets, comminute them to a fine powder in a suitable mortar, or add Azathioprine powder to the mortar. Add about 10 mL of the Vehicle, and mix to a uniform paste. Add the Vehicle in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient Vehicle to bring to final volume, and mix well.

[CAUTION—Avoid skin contact or inhalation of azathioprine by using protective gloves and a fume hood or surgical mask.]

### ASSAY

#### • PROCEDURE

**Mobile phase:** Dissolve 1.1 g of sodium-1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust with 1 N hydrochloric acid to a pH of 3.5.

**Standard solution:** Transfer 25 mg of USP Azathioprine RS to a 50-mL volumetric flask. Add 15 mL of methanol and 0.5 mL of ammonium hydroxide to the flask, swirl, and sonicate for 2 min. Dilute with methanol to volume. Transfer 10 mL of this solution to a 50-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with Mobile phase to volume.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 2 mL/min

Injection volume: 20 μL

### System suitability

Sample: Standard solution

[NOTE—The retention time for the azathioprine peak is about 4 min.]

### Suitability requirements

Relative standard deviation: NMT 1.3% for replicate injections

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of azathioprine (C<sub>9</sub>H<sub>7</sub>N<sub>7</sub>O<sub>2</sub>S) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Azathioprine RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of azathioprine in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

### SPECIFIC TESTS

- **PH (791):** 3.8–4.8

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at room temperature, or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded when stored at room temperature, or in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken before use, and to state the Beyond-Use Date.
- **USP REFERENCE STANDARDS (11)**  
USP Azathioprine RS

## Azathioprine Tablets

### DEFINITION

Azathioprine Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of azathioprine (C<sub>9</sub>H<sub>7</sub>N<sub>7</sub>O<sub>2</sub>S).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Standard solution:** 20 mg/mL of USP Azathioprine RS in 6 N ammonium hydroxide

**Sample solution:** Nominally 20 mg/mL of azathioprine in 6 N ammonium hydroxide, from powdered Tablets. Filter the solution.

### Chromatographic system

Adsorbent: 0.25-mm layer of microcrystalline cellulose

Application volume: 5 μL

Developing solvent system: Butyl alcohol saturated with 6 N ammonium hydroxide



**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in the chapter.

**Acceptance criteria:** Meet the requirements

**ASSAY****• PROCEDURE**

**Mobile phase:** Dissolve 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust the solution with 1 N hydrochloric acid to a pH of 3.5. Filter the solution through a 0.8- $\mu$ m, solvent-resistant membrane, and degas.

**Standard stock solution:** 0.5 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add methanol equivalent to 30% of the final volume and ammonium hydroxide equivalent to 1% of the final volume, swirl, and sonicate for 2 min. Dilute with methanol to volume.

**Standard solution:** 0.1 mg/mL of USP Azathioprine RS in water prepared as follows. Transfer 10.0 mL of *Standard stock solution* into a 50-mL volumetric flask, and dilute with water to volume.

**Sample stock solution:** 0.5 mg/mL of azathioprine prepared as follows. Transfer an equivalent to 50 mg of azathioprine, from powdered Tablets (NLT 20), into a 100-mL volumetric flask. Add 25 mL of methanol and 1.0 mL of ammonium hydroxide to the flask, swirl, and sonicate for 2 min. Dilute with methanol to volume. Allow the excipients to settle.

**Sample solution:** 0.1 mg/mL of azathioprine in water prepared as follows. Transfer 10.0 mL of the *Sample stock solution* to a 50-mL volumetric flask, and dilute with water to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 800 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of azathioprine ( $C_9H_7N_7O_2S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of azathioprine from the *Sample solution*

$r_s$  = peak response of azathioprine from the *Standard solution*

$C_s$  = concentration of USP Azathioprine RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of azathioprine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–107.0%

**PERFORMANCE TESTS****• DISSOLUTION (711)**

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Standard solution:** USP Azathioprine RS in *Medium*

**Sample solutions:** Sample per *Dissolution* (711). Filter, and dilute with *Medium*, if necessary, to a concentration that is similar to that of the *Standard solution*.

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** At maximum absorbance of about 280 nm

**Tolerances:** NLT 75% (Q) of the labeled amount of azathioprine ( $C_9H_7N_7O_2S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Protect from light.

- **USP REFERENCE STANDARDS (11)**

USP Azathioprine RS

**Azathioprine Sodium for Injection****DEFINITION**

Azathioprine Sodium for Injection is a sterile solid prepared by the freeze-drying of an aqueous solution of Azathioprine and Sodium Hydroxide. It contains NLT 93.0% and NMT 107.0% of the labeled amount of azathioprine ( $C_9H_7N_7O_2S$ ).

**IDENTIFICATION**

- **A.** The principal spot from the *Sample solution* shows the same  $R_f$  value as that obtained from *Standard solution A* in the test for *Limit of Mercaptopurine*.

**Add the following:**

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. ▲ USP40

**ASSAY****Change to read:****• PROCEDURE**

**▲ Solution A:** 1.6 g/L of sodium 1-heptanesulfonate in water

**Mobile phase:** Methanol and *Solution A* (30:70). Adjust with 1 N hydrochloric acid to a pH of  $3.5 \pm 0.1$ .

**Standard stock solution:** 0.5 mg/mL of USP Azathioprine RS prepared as follows. Transfer USP Azathioprine RS to a suitable volumetric flask. Add 25% of the flask volume of methanol and 1% of ammonium hydroxide to the flask, swirl, and sonicate for 2 min or until dissolved. Dilute with methanol to volume.

**Standard solution:** 0.1 mg/mL of USP Azathioprine RS in water from the *Standard stock solution*

**Sample solution:** Nominally equivalent to 0.1 mg/mL of azathioprine in water from Azathioprine Sodium for Injection

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-μm packing L1

Flow rate: 1.8 mL/min

Injection volume: 10 μL

System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2

Relative standard deviation: NMT 1.0%

Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of azathioprine (C<sub>9</sub>H<sub>7</sub>N<sub>7</sub>O<sub>2</sub>S) in the portion of Azathioprine Sodium for Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of azathioprine from the Sample solution

$r_s$  = peak response of azathioprine from the Standard solution

$C_s$  = concentration of USP Azathioprine RS in the Standard solution (mg/mL)

$C_u$  = nominal concentration of azathioprine in the Sample solution (mg/mL)<sub>▲USP40</sub>

Acceptance criteria: 93.0%–107.0%

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

**IMPURITIES**• **LIMIT OF MERCAPTOPURINE**

Standard solution A: 10 mg/mL of USP Azathioprine RS in dimethylformamide

Standard solution B: 100 μg/mL of USP Mercaptopurine RS in dimethylformamide

Sample solution: 10 mg/mL of Azathioprine Sodium for Injection in dimethylformamide

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Mode: TLC

Adsorbent: 0.25-mm layer of microcrystalline cellulose

Application volume: 5 μL for Standard solution A and the Sample solution, and 15 μL for Standard solution B

Developing solvent system: Butyl alcohol saturated with 5 N ammonium hydroxide

Analysis

Samples: Standard solution A, Standard solution B, and Sample solution

Proceed as directed in the chapter. Apply the Samples at points 2 cm from the bottom edge of a TLC plate. Allow the spots to dry, and develop the chromatogram in a suitable chamber until the solvent front has moved 15 cm from the point of application. Remove the plate, air-dry, and locate the spots by viewing under short- and long-wavelength UV light.

Acceptance criteria: 3.0%; any spot from the Sample solution, other than the principal spot, is not more intense than the spot from Standard solution B.

**SPECIFIC TESTS**

Delete the following:

▲• **COMPLETENESS OF SOLUTION (641)**

Sample: 1 container of Azathioprine Sodium for Injection

Acceptance criteria: The contents of the Sample are soluble in 10 mL of water to give a clear, bright yellow solution, essentially free from foreign matter.<sub>▲USP40</sub>

• **pH (791)**

Sample solution: The contents of one container dissolved in 10 mL of water

Acceptance criteria: 9.8–11.0

- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 1.0 USP Endotoxin Unit/mg of azathioprine.

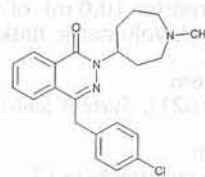
- **WATER DETERMINATION (921), Method I:** NMT 7.0%, when the Test Preparation is prepared as directed for a hygroscopic specimen

- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products (1)*.

**ADDITIONAL REQUIREMENTS**

Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements (659)*, *Injection Packaging, Packaging for constitution* (CN 1-May-2017), at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Azathioprine RS  
USP Endotoxin RS  
USP Mercaptopurine RS

**Azelastine Hydrochloride**

C<sub>22</sub>H<sub>24</sub>ClN<sub>3</sub>O · HCl 418.36  
1(2*H*)-Phthalazinone, 4-[(4-chlorophenyl)methyl]-2-(hexahydro-1-methyl-1*H*-azepin-4-yl), monohydrochloride; 4-(*p*-Chlorobenzyl)-2-(hexahydro-1-methyl-1*H*-azepin-4-yl)-1(2*H*)-phthalazinone monohydrochloride [79307-93-0].

**DEFINITION**

Azelastine Hydrochloride contains NLT 99.0% and NMT 101.0% of azelastine hydrochloride (C<sub>22</sub>H<sub>24</sub>ClN<sub>3</sub>O · HCl), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the Sample solution corresponds to that of the Identification solution, as obtained in the test for *Organic Impurities*.
- **C. IDENTIFICATION TESTS—GENERAL (191), Chloride:** Meets the requirements

**ASSAY**• **PROCEDURE**

Sample: 300 mg

Blank: 5 mL of anhydrous formic acid and 30 mL of acetic anhydride

Titrimetric system

(See *Titrimetry (541)*.)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the endpoint has been reached. Dissolve the Sample in 5 mL of anhydrous formic acid, and add 30 mL of acetic anhydride. Titrate with Titrant.



Calculate the percentage of azelastine hydrochloride ( $C_{22}H_{24}ClN_3O \cdot HCl$ ) in the portion of Azelastine Hydrochloride taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

$V_S$  = Titrant volume consumed by the Sample (mL)

$V_B$  = Titrant volume consumed by the Blank (mL)

$N$  = actual normality of the Titrant (mEq/mL)

$F$  = equivalency factor, 41.84 mg/mEq

$W$  = weight of the Sample (mg)

Acceptance criteria: 99.0%–101.0% on the dried basis

## IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

## Delete the following:

- **HEAVY METALS** (231), Method II: NMT 20 ppm (Official 1-

Jan-2018)

- **ORGANIC IMPURITIES**

**Dilute phosphoric acid:** 115 g/L of phosphoric acid in water

**Buffer:** 2.92 g/L of octanesulfonic acid sodium salt and 0.92 g/L of monobasic potassium phosphate in water.

Adjust with *Dilute phosphoric acid* to a pH of 3.0–3.1.

**Mobile phase:** Acetonitrile and Buffer (260:740)

**Diluent:** Acetonitrile and water (45:55)

**Identification solution:** 2.5 mg/mL of USP Azelastine Hydrochloride RS in *Diluent*. [NOTE—This solution is used for *Identification test B*.]

**System suitability stock solution:** 0.5 mg/mL each of USP Azelastine Related Compound B RS, USP Azelastine Related Compound D RS, and USP Azelastine Related Compound E RS in acetonitrile

**System suitability solution:** 50 µg/mL each of USP Azelastine Related Compound B RS, USP Azelastine Related Compound D RS, and USP Azelastine Related Compound E RS from the *System suitability stock solution* and 2.5 mg/mL of USP Azelastine Hydrochloride RS in *Diluent*

**Standard stock solution:** 50 µg/mL of USP Azelastine Hydrochloride RS in acetonitrile

**Standard solution:** 2.5 µg/mL of USP Azelastine Hydrochloride RS in *Diluent*

**Sample solution:** 2.5 mg/mL of Azelastine Hydrochloride in *Diluent*

## Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; 10-µm packing L10

**Column temperature:** 30°

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

**Run time:** 2.4 times the retention time of azelastine

## System suitability

**Samples:** *System suitability solution* and *Standard solution*

## Suitability requirements

**Resolution:** NLT 4.0 between azelastine related compound B and azelastine related compound D; NLT 1.5 between azelastine related compound D and azelastine; NLT 1.5 between azelastine and azelastine related compound E, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

## Analysis

**Samples:** *Identification solution*, *Standard solution*, and *Sample solution*

Calculate the percentage of each impurity in the portion of Azelastine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak area of each impurity from the *Sample solution*

$r_S$  = peak area of azelastine from the *Standard solution*

$C_S$  = concentration of USP Azelastine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Azelastine Hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. [NOTE—Disregard peaks that are less than 0.05% of the azelastine peak.]

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Benzohydrazide	0.2	0.38	0.1
Azelastine related compound B <sup>a</sup>	0.3	0.22	0.1
Chlorophenylacetylbenzoic acid <sup>b</sup>	0.4	0.45	0.1
Azelastine related compound D <sup>c</sup>	0.6	1.2	0.1
Azelastine	1.0	1.0	—
Azelastine related compound E <sup>d</sup>	1.4	0.48	0.1
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.2

<sup>a</sup> N'-(1-Methylazepan-4-yl)benzohydrazide; also known as 1-Benzoyl-2-[(4RS)-1-methylhexahydro-1H-azepin-4-yl]diazane.

<sup>b</sup> 2-[2-(4-Chlorophenyl)acetyl]benzoic acid.

<sup>c</sup> 4-(4-Chlorobenzyl)phthalazin-1(2H)-one.

<sup>d</sup> 3-(4-Chlorobenzylidene)isobenzofuran-1(3H)-one.

## SPECIFIC TESTS

- **LOSS ON DRYING** (731)

**Analysis:** Dry at 105° to constant weight.

**Acceptance criteria:** NMT 1.0%

- **ACIDITY OR ALKALINITY**

**Sample solution:** 10 mg/mL of Azelastine Hydrochloride in water

**Analysis:** Add 0.2 mL of bromothymol blue TS to 10 mL of the *Sample solution*.

**Acceptance criteria:** NMT 0.1 mL of 0.01 M hydrochloric acid or 0.01 M sodium hydroxide is required to produce a color change.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Protect from light and moisture. Store at controlled room temperature.

- **USP REFERENCE STANDARDS** (11)

USP Azelastine Hydrochloride RS

USP Azelastine Related Compound B RS

N'-(1-Methylazepan-4-yl)benzohydrazide.

$C_{14}H_{21}N_3O$  247.34

USP Azelastine Related Compound D RS

4-(4-Chlorobenzyl)phthalazin-1(2H)-one.

$C_{15}H_{11}ClN_2O$  270.71

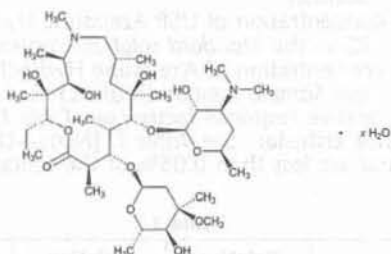
USP Azelastine Related Compound E RS

3-(4-Chlorobenzylidene)isobenzofuran-1(3H)-one.

$C_{15}H_9ClO_2$  256.68



## Azithromycin



$C_{38}H_{72}N_2O_{12}$  748.98

$C_{38}H_{72}N_2O_{12} \cdot H_2O$  767.00

$C_{38}H_{72}N_2O_{12} \cdot 2H_2O$  785.02

1-Oxa-6-azacyclopentadecan-15-one, 13-[(2,6-dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-, [2R-(2R\*,3S\*,4R\*,5R\*,8R\*,10R\*,11R\*,12S\*,13S\*,14R\*)]; (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one;

9-Deoxo-9a-aza-9a-methyl-9a-homoerythromycin A

Anhydrous [83905-01-5].

Monohydrate [121470-24-4].

Dihydrate [117772-70-0].

### DEFINITION

Azithromycin is anhydrous or contains one or two molecules of water of hydration. It contains the equivalent of NLT 945  $\mu$ g and NMT 1030  $\mu$ g of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) per mg, calculated on the anhydrous basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION (197K):** If a difference appears in the IR spectra of the analyte and the Standard, dissolve equal portions of the test specimen and the USP Reference Standard in equal volumes of methanol. Evaporate the solutions to dryness on a water bath, and dry at 80° for 30 min under vacuum. Perform the test on the residues.
- B.** The retention time of the azithromycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Solution A:** 10 M Potassium hydroxide

**Solution B:** 6.7 g/L of dibasic potassium phosphate adjusted with *Solution A* to a pH of 11.0

**Solution C:** 6.7 g/L of dibasic potassium phosphate adjusted with phosphoric acid to a pH of 8.0

**Mobile phase:** Acetonitrile and *Solution B* (60:40)

**Diluent:** Acetonitrile and *Solution C* (60:40)

**System suitability solution:** 0.5 mg/mL each of USP Azithromycin RS and USP Azaerythromycin A RS prepared as follows. Dissolve USP Azithromycin RS and USP Azaerythromycin A RS first in acetonitrile, using 5% of the final volume, and then dilute with *Diluent* to volume.

**Standard solution:** 0.53 mg/mL of USP Azithromycin RS prepared as follows. Dissolve USP Azithromycin RS first in acetonitrile, using 2% of the final volume, and then dilute with *Diluent* to volume.

**Sample solution:** 0.53 mg/mL of Azithromycin prepared as follows. Dissolve Azithromycin first in acetonitrile, using 2% of the final volume, and then dilute with *Diluent* to volume.

trile, using 2% of the final volume, and then dilute with *Diluent* to volume.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L67

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for azaerythromycin A and azithromycin are 0.7 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 3.0 between azaerythromycin A and azithromycin, *System suitability solution*

**Tailing factor:** 0.8–1.5 for azithromycin, *Standard solution*

**Relative standard deviation:** NMT 1.10% for azithromycin, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in  $\mu$ g, of azithromycin

( $C_{38}H_{72}N_2O_{12}$ ) in each mg of Azithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution*

$C_U$  = concentration of Azithromycin in the *Sample solution*

$P$  = potency of USP Azithromycin RS ( $\mu$ g/mg of azithromycin)

**Acceptance criteria:** 945–1030  $\mu$ g/mg on the anhydrous basis

### IMPURITIES

- RESIDUE ON IGNITION (281):** NMT 0.3%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

### Delete the following:

- HEAVY METALS, Method II (231):** NMT 25 ppm (Official 1, Jan-2018)

### ORGANIC IMPURITIES, PROCEDURE 1

Use *Organic Impurities, Procedure 1* when the impurity profile includes erythromycin A oxime and erythromycin A iminoether.

Use water that has a resistivity of NLT 18 Mohm-cm.

**Solution A:** 20 mM Dibasic potassium phosphate

**Mobile phase:** Acetonitrile and *Solution A* (250:750).

Adjust with 5 M potassium hydroxide to a pH of 10.55  $\pm$  0.05.

**Standard stock solution:** 45  $\mu$ g/mL of USP Desosaminylazithromycin RS, 105  $\mu$ g/mL of USP *N*-Demethylazithromycin RS, 150  $\mu$ g/mL of USP Azaerythromycin A RS, and 160  $\mu$ g/mL of USP Azithromycin RS in acetonitrile. Sonicate as necessary to dissolve.

**Standard solution:** 0.9  $\mu$ g/mL of USP Desosaminylazithromycin RS, 2.1  $\mu$ g/mL of USP *N*-Demethylazithromycin RS, 3.0  $\mu$ g/mL of USP Azaerythromycin A RS, and 3.2  $\mu$ g/mL of USP Azithromycin RS from the *Standard stock solution* in *Mobile phase*

**Sample solution:** 0.33 mg/mL of Azithromycin prepared as follows. Transfer a suitable amount of Azithromycin to a suitable volumetric flask. Add acetonitrile, using 5% of the final volume, and sonicate as necessary to dissolve. Dilute with *Mobile phase* to volume.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Amperometric electrochemical

Detector type: Dual glassy carbon electrodes

Detector mode: Oxidative screen mode

Detector settings

Electrode 1: +0.70V

Electrode 2: +0.82V

Column: 4.6-mm × 15-cm; 3-μm packing L49

Temperatures

Detector preheater: 28°

Autosampler: 5°

Flow rate: 1 mL/min

Injection volume: 50 μL

**System suitability**Sample: *Standard solution***Suitability requirements**

Resolution: NLT 3.0 between azithromycin and azaerythromycin A

Tailing factor: NMT 2.0 for azithromycin; NMT 2.5 for *N*-demethylazithromycinRelative standard deviation: NMT 10.0% for azithromycin, azaerythromycin A, *N*-demethylazithromycin, and desosaminylazithromycin**Analysis**Samples: *Standard solution* and *Sample solution*Record the *Sample solution* chromatograms for NLT 3.3 times the retention time of the azithromycin peak.Calculate the percentages of desosaminylazithromycin, *N*-demethylazithromycin, and azaerythromycin A in the portion of Azithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

 $r_U$  = peak area of the relevant analyte from the *Sample solution* $r_S$  = peak area of the relevant analyte from the *Standard solution* $C_S$  = concentration of the appropriate USP Reference Standard in the *Standard solution* (μg/mL) $C_U$  = concentration of the *Sample solution* (mg/mL) $F$  = conversion factor, 0.001 mg/μg

Calculate the percentages of other related substances in the portion of Azithromycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

 $r_U$  = peak area of each additional impurity from the *Sample solution* $r_S$  = peak area of the azithromycin peak from the *Standard solution* $C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (μg/mL) $C_U$  = concentration of the *Sample solution* (mg/mL) $F$  = conversion factor, 0.001 mg/μg

Acceptance criteria: See Table 1.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Erythromycin A iminoether <sup>a</sup>	0.19	0.5
Desosaminylazithromycin <sup>b</sup>	0.29	0.3
Erythromycin A oxime <sup>c</sup>	0.37	0.5
<i>N</i> -Demethylazithromycin <sup>d</sup>	0.49	0.7
Azaerythromycin A <sup>e</sup>	0.80	1.0
Azithromycin	1.0	—
3-Deoxyazithromycin (azithromycin B) <sup>f</sup>	2.33	1.0
Total impurities	—	3.0

<sup>a</sup> (3*R*,4*R*,5*S*,6*R*,9*R*,10*S*,11*S*,12*R*,13*S*,15*R*,2*Z*)-12-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-6-ethyl-4,5-dihydroxy-10-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-3,5,9,11,13,15-hexamethyl-7,16-dioxo-2-azabicyclo[11.2.1]hexadec-1-en-8-one.<sup>b</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.<sup>c</sup> (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*,E)-6-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-14-ethyl-7,12,13-trihydroxy-4-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethyloxacyclotetradecan-2-one.<sup>d</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.<sup>e</sup> 9-Deoxy-9a-aza-9a-homoerythromycin A; 6-De-methylazithromycin.<sup>f</sup> (2*R*,3*R*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.**• ORGANIC IMPURITIES, PROCEDURE 2**Use *Organic Impurities, Procedure 1* when the impurity profile includes erythromycin A oxime and erythromycin A iminoether.**Solution A:** 1.8 mg/mL of anhydrous dibasic sodium phosphate in water. Adjust with 1 N sodium hydroxide or 10% phosphoric acid to a pH of 8.9.**Solution B:** Acetonitrile and methanol (3:1)**Solution C:** 1.73 mg/mL of monobasic ammonium phosphate. Adjust with ammonia TS to a pH of 10.0 ± 0.05.**Solution D:** Methanol, acetonitrile, and *Solution C* (7:6:7)

Mobile phase: See Table 2.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

**System suitability solution:** 0.0165 mg/mL of USP Azithromycin Related Compound F RS and 0.027 mg/mL of USP Desosaminylazithromycin RS in *Solution D***Standard solution:** 86 μg/mL of USP Azithromycin RS in *Solution D***Sample solution:** 8.6 mg/mL of Azithromycin in *Solution D***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)



**Mode:** LC**Detector:** UV 210 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Column temperature:** 60°**Flow rate:** 1 mL/min**Injection volume:** 50 μL**System suitability****Samples:** System suitability solution and Standard solution**Suitability requirements****Tailing factor:** 0.8–1.5, Standard solution**Peak-to-valley ratio:** NLT 1.4, System suitability solution. Calculate the peak-to-valley ratio as follows:

$$\text{Result} = H_p/H_v$$

 $H_p$  = height above the baseline of the desosaminylazithromycin peak $H_v$  = height above the baseline of the lowest point of the curve separating the desosaminylazithromycin and azithromycin related compound F peaks**Analysis****Samples:** Standard solution and Sample solution

Calculate the percentage of each related compound in the portion of Azithromycin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F_1 \times (100/F_2)$$

 $r_u$  = peak response of each impurity from the Sample solution $r_s$  = peak response of azithromycin from the Standard solution $C_s$  = concentration of USP Azithromycin RS in the Standard solution (mg/mL) $C_u$  = concentration of Azithromycin in the Sample solution (mg/mL) $P$  = potency of USP Azithromycin RS (μg/mg of azithromycin) $F_1$  = conversion factor, 0.001 mg/μg $F_2$  = relative response factor (see Table 3)**Acceptance criteria:** See Table 3. Disregard peaks eluting before azithromycin N-oxide and after 3-deoxyazithromycin (azithromycin B). Disregard peaks with a response less than 0.1 times the response of the azithromycin peak in the Standard solution (0.1%).**Table 3**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Azithromycin N-oxide <sup>a</sup>	0.29	0.43	0.5
3'-(N,N-Didemethyl)-3'-N-formylazithromycin <sup>b</sup>	0.37	1.7	0.5
3'-(N,N-Didemethyl) azithromycin (aminoazithromycin) <sup>c</sup>	0.43	1.0	0.5
Azithromycin related compound F <sup>d,e</sup>	0.51	3.8	0.5
Desosaminylazithromycin <sup>f</sup>	0.54	1.0	0.3
3'-N-[[4-(Acetylamino)phenyl]sulfonyl]-3',3'-didemethylazithromycin <sup>g</sup>	0.55	12	0.15
N-Demethylazithromycin <sup>h</sup>	0.61	1.0	0.7
Azithromycin C (3'-O-demethylazithromycin) <sup>i</sup>	0.73	1.0	0.5
3'-De(dimethylamino)-3'-oxoazithromycin <sup>j</sup>	0.76	1.5	0.5
3'-N-[[4-(Acetylamino)phenyl]sulfonyl]-3'-demethylazithromycin <sup>k</sup>	0.79	10	0.5
Azaerythromycin A <sup>l</sup>	0.83	1.0	0.5
Azithromycin impurity P <sup>m</sup>	0.92	1.0	0.2
Azithromycin	1.0	—	—

<sup>a</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylazino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>b</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>c</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>d</sup> 3'-N-Demethyl-3'-N-formylazithromycin; (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(N-methyl)formamido-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>e</sup> The system may resolve two rotamers of azithromycin related compound F. The sum of the two rotamers is reported.<sup>f</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>g</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[N-(4-acetamidophenylsulfonyl)amino]-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>h</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>i</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>j</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>k</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[N-(4-acetamidophenylsulfonyl)-N-methylamino]-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>l</sup> 9-Deoxy-9a-aza-9a-homoerythromycin A; 6-Demethylazithromycin.<sup>m</sup> Specified unidentified impurity.<sup>n</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-propyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>o</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[N-(4-methylphenylsulfonyl)-N-methylamino]-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.<sup>p</sup> (2R,3R,4S,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.



Table 3 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
2-Desethyl-2-propylazithromycin <sup>a</sup>	1.23	1.0	0.5
3'-N-Demethyl-3'-N-[(4-methylphenyl)sulfonyl]azithromycin <sup>a</sup>	1.26	5	0.5
3-Deoxyazithromycin (azithromycin B) <sup>a</sup>	1.31	1.0	1.0
Any individual, unidentified impurity	—	1.0	0.2
Total impurities	—	—	3.0

<sup>a</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>b</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>c</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>d</sup> 3'-N-Demethyl-3'-N-formylazithromycin; (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(N-methyl)formamido-3,4,6-trideoxy- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>e</sup> The system may resolve two rotamers of azithromycin related compound F. The sum of the two rotamers is reported.

<sup>f</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>g</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(N-(4-acetamidophenyl)sulfonyl)amino]-3,4,6-trideoxy- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>h</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>i</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>j</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3,3-dimethyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>k</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(N-(4-acetamidophenyl)sulfonyl)-N-methylamino]-3,4,6-trideoxy- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>l</sup> 9-Deoxy-9a-aza-9a-homoerythromycin A; 6-Demethylazithromycin.

<sup>m</sup> Specified unidentified impurity.

<sup>n</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-propyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>o</sup> (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(N-(4-methylphenyl)sulfonyl)-N-methylamino]-3,4,6-trideoxy- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>p</sup> (2R,3R,4S,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

## SPECIFIC TESTS

- OPTICAL ROTATION, Specific Rotation (781S):**  $-45^{\circ}$  to  $-49^{\circ}$ , at  $20^{\circ}$

Sample solution: 20 mg/mL in dehydrated alcohol

- CRYSTALLINITY (69S):** Meets the requirements except, where it is labeled as amorphous, most of the particles do not exhibit birefringence and extinction positions
- PH (791):** 9.0–11.0

Sample stock solution: 4 mg/mL in methanol

Sample solution: 2 mg/mL obtained by mixing equal volumes of Sample stock solution and water

- WATER DETERMINATION, Method I (921)**

Where it is labeled as anhydrous: NMT 2.0%

Where it is labeled as the dihydrate: 4.0%–5.0%

Where it is labeled as the monohydrate: 1.8%–4.0%, except that it may be 4.0%–6.5% when the requirements of the Loss on Drying test are met

- LOSS ON DRYING:** Where it is labeled as Azithromycin monohydrate and has a water content of 4.0%–6.5% (see Thermal Analysis (891))

[NOTE—The quantity taken for this procedure may be adjusted, if necessary, for instrument sensitivity.]

**Analysis:** Determine the percentage of volatile substances by thermogravimetric analysis in an appropriately calibrated instrument, using about 10 mg of Azithromycin. Heat the specimen at the rate of  $10^{\circ}/\text{min}$  between ambient temperature and  $150^{\circ}$  in an atmosphere of nitrogen at a constant flow rate of about 35 mL/min. From the thermogram plot the derivatives of the loss on drying (percent loss/min), and identify the inflection points of the two weight loss steps at about  $70^{\circ}$  and  $130^{\circ}$ .

**Acceptance criteria:** It loses NMT 4.5% of its weight between ambient temperature and the inflection point at about  $70^{\circ}$ , and 1.8%–2.6% between the inflection

point at about  $70^{\circ}$  and the inflection point at about  $130^{\circ}$ .

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers.
- LABELING:** Label it to indicate whether it is anhydrous, or the monohydrate, or the dihydrate. The amorphous form is so labeled. Where the quantity of azithromycin is indicated in the labeling of any preparation containing Azithromycin, this shall be understood to be in terms of anhydrous azithromycin ( $\text{C}_{38}\text{H}_{70}\text{N}_2\text{O}_{12}$ ). The labeling states with which Organic Impurities procedure the article complies, if other than Procedure 1.

## USP REFERENCE STANDARDS (11)

USP Azaerythromycin A RS

9-Deoxy-9a-aza-9a-homoerythromycin A;

6-Demethylazithromycin.

$\text{C}_{37}\text{H}_{70}\text{N}_2\text{O}_{12}$  734.96

USP Azithromycin RS

USP Azithromycin Related Compound F RS

3'-N-Demethyl-3'-N-formylazithromycin; (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(N-methyl)formamido-3,4,6-trideoxy- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

$\text{C}_{38}\text{H}_{70}\text{N}_2\text{O}_{13}$  762.97

USP N-Demethylazithromycin RS

(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

$\text{C}_{37}\text{H}_{70}\text{N}_2\text{O}_{12}$  734.96



USP Desosaminylazithromycin RS  
(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.  
 $C_{30}H_{58}N_2O_9$  590.79

## Azithromycin Capsules

### DEFINITION

Azithromycin Capsules contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ).

### IDENTIFICATION

- A. The retention time of the azithromycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

[NOTE—Use water that has a resistivity of NLT 18 Mohm-cm.]

**Mobile phase:** Dissolve 5.8 g of monobasic potassium phosphate in 2130 mL of water, and add 870 mL of acetonitrile. Adjust with about 6 mL of 10 N potassium hydroxide to a pH of  $11.0 \pm 0.1$ , and pass through a suitable filter.

**Standard stock solution:** 0.165 mg/mL of USP Azithromycin RS in acetonitrile. Swirl, and sonicate as necessary.

**Standard solution:** 3.3 µg/mL of USP Azithromycin RS from the *Standard stock solution* in *Mobile phase*

**System suitability stock solution:** 0.16 mg/mL of USP Azaerythromycin A RS in acetonitrile and *Mobile phase* (1:9). Dissolve first in acetonitrile, using 10% of the final volume. Swirl, and sonicate to dissolve. Dilute with *Mobile phase* to volume.

**System suitability solution:** 3.2 µg/mL of azaerythromycin A from the *System suitability stock solution* and 3.3 µg/mL of azithromycin from the *Standard stock solution* in *Mobile phase*

**Sample stock solution:** Remove, as completely as possible, the contents of NLT 20 Capsules. Prepare a 1-mg/mL solution of anhydrous azithromycin in acetonitrile. Dissolve a portion of the mixed Capsule contents first in 70% of the final volume of acetonitrile, and shake by mechanical means for 30 min. Dilute with acetonitrile to volume. Place 40 mL of the resulting suspension in a centrifuge tube, and centrifuge. Use the supernatant to prepare the *Sample solution*.

**Sample solution:** 3.2 µg/mL of azithromycin from the *Sample stock solution* in *Mobile phase*

#### Chromatographic system

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** Amperometric electrochemical detector

**Electrode:** Dual glassy carbon electrodes

**Mode:** Oxidative screen mode

**Electrode 1:**  $+0.70 \pm 0.05$  V

**Electrode 2:**  $+0.82 \pm 0.05$  V

**Background current:**  $85 \pm 15$  nanoamperes

#### Columns

**Guard:** 4.6-mm × 5-cm; 5-µm packing L29

**Analytical:** 4.6-mm × 15-cm; 5-µm packing L29 or 3-µm packing L49 without the guard column

**Flow rate:** 1.5 mL/min

**Injection size:** 50 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for azaerythromycin A and azithromycin with the L29 column are 0.7 and 1.0, respectively; the relative retention times for azaerythromycin A and azithromycin with the L49 column are 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*

**Column efficiency:** NLT 1000 theoretical plates, *Standard solution*

**Tailing factor:** 0.9–1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of azithromycin in the *Sample solution* (µg/mL)

$P$  = potency of azithromycin in USP Azithromycin RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION (711)

[NOTE—Use water that has a resistivity of NLT 18 Mohm-cm.]

**Medium:** pH 6.0 sodium phosphate buffer (Prepare 6 L of 0.1 M dibasic sodium phosphate. Adjust with about 40 mL of hydrochloric acid to a pH of  $6.0 \pm 0.05$ , and add 600 mg of trypsin); 900 mL

**Apparatus 2:** 100 rpm

**Time:** 45 min

**Mobile phase, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Standard stock solution:** 0.3 mg/mL of USP Azithromycin RS in *Medium*. Sonicate briefly to dissolve.

**Standard solution:** 3.84 µg/mL of azithromycin from the *Standard stock solution* in *Mobile phase*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.5-µm or finer pore size. Transfer 2.0 mL of the filtrate to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume. Transfer 4.0 mL of this solution to a second 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) dissolved using the procedure in the Assay, making any necessary modifications.

Calculate the percentage of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Capsule)

$D$  = dilution factor of the *Sample solution*

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 75% (Q) of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements



**SPECIFIC TESTS**

- **WATER DETERMINATION**, Method I (921): NMT 5.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in well-closed containers. Where packaged in unit-of-use containers, each container contains six 250-mg Capsules, and the label indicates the intended sequential day of use for each Capsule.
- **USP REFERENCE STANDARDS (11)**  
USP Azaerythromycin A RS  
USP Azithromycin RS

**Azithromycin for Injection****DEFINITION**

Azithromycin for Injection is a sterile, dry mixture of azithromycin and a suitable stabilizing agent. It contains NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ).

**IDENTIFICATION**

- **A**. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer**: 6.7 mg/mL of dibasic potassium phosphate in water

**Mobile phase**: Acetonitrile and *Buffer* (52:48). Adjust with 10 N potassium hydroxide to a pH of  $11.0 \pm 0.1$ .

**Diluent**: Acetonitrile and water (52:48)

**System suitability solution**: 1 mg/mL each of USP Azaerythromycin A RS and USP Azithromycin RS in a mixture of acetonitrile and water (52:48). Dissolve first in acetonitrile, and then dilute with water to volume.

**Standard solution**: 1 mg/mL of USP Azithromycin RS in a mixture of acetonitrile and water (52:48). Dissolve first in acetonitrile, and dilute with water to volume.

**Sample solution**: Equivalent to 1 mg/mL of azithromycin from Azithromycin for Injection in *Diluent* prepared as follows. Reconstitute 3 vials individually as directed in the labeling. Mix the contents of all the reconstituted vials. Dilute a portion of the mixture with *Diluent*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode**: LC

**Detector**: UV 215 nm

**Columns**

**Guard**: 4.6-mm  $\times$  1-cm; 5- $\mu$ m packing L67

**Analytical**: 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L67

**Temperature**

**Column**: 40°

**Autosampler**: 15°

**Flow rate**: 1 mL/min

**Injection size**: 15  $\mu$ L

**System suitability**

**Samples**: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for azaerythromycin A and azithromycin are 0.68 and 1.0, respectively.]

**Suitability requirements**

**Resolution**: NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*

**Tailing factor**: NLT 0.9 and NMT 1.5, *Standard solution*

**Relative standard deviation**: NMT 2%, *Standard solution*

**Analysis**

**Samples**: *Standard solution* and *Sample solution*  
Calculate the percentage of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) in the portion of Azithromycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of azithromycin in the *Sample solution* (mg/mL)

$P$  = potency of USP Azithromycin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria**: 90.0%–110.0%

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

**IMPURITIES**

[NOTE—The test for *Limit of Azithromycin N-Oxide*, *Desosaminylazithromycin*, and *N-Demethylazithromycin* does not quantify aminoazithromycin, formamido analog, methylformamido analog, and 3'-de(dimethylamino)-3'-oxoazithromycin. If these impurities are part of the impurity profile, the *Limit of Aminoazithromycin*, *Formamido Analog*, *Methylformamido Analog*, and 3'-*De(dimethylamino)-3'-oxoazithromycin* test is recommended in addition to the test for *Limit of Azithromycin N-Oxide*, *Desosaminylazithromycin*, and *N-Demethylazithromycin*.]

- **LIMIT OF AZITHROMYCIN N-OXIDE, DESOSAMINYLAZITHROMYCIN, AND N-DEMETHYLAZITHROMYCIN**

**Buffer**: 3.5 g/L of dibasic potassium phosphate

**Mobile phase**: Acetonitrile and *Buffer* (23:77). Adjust with 5 N potassium hydroxide to a pH of  $10.55 \pm 0.05$ .

**Standard stock solution**: 50  $\mu$ g/mL of USP Azithromycin N-oxide RS, 45  $\mu$ g/mL of USP Desosaminylazithromycin RS, and 160  $\mu$ g/mL each of USP *N-Demethylazithromycin* RS and USP Azithromycin RS in acetonitrile. Sonicate if necessary to dissolve.

**Standard solution**: 1  $\mu$ g/mL of azithromycin N-oxide, 0.9  $\mu$ g/mL of desosaminylazithromycin, 3.2  $\mu$ g/mL each of *N-demethylazithromycin* and azithromycin from *Standard stock solution* in *Mobile phase*

**Sample solution**: Equivalent to 0.3 mg/mL of azithromycin in *Mobile phase* from Azithromycin for Injection

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode**: LC

**Detector**: Amperometric electrochemical detector

**Electrode**: Dual series glassy carbon electrodes

**Mode**: Oxidative screen mode

**Electrode 1**:  $+0.70 \pm 0.05$ V

**Electrode 2**:  $+0.82 \pm 0.05$ V

**Background current**:  $95 \pm 25$  nanoamperes

**Column**: 4.6-mm  $\times$  15-cm; 3- $\mu$ m packing L49

**Flow rate**: 1 mL/min

**Injection size**: 50  $\mu$ L

**Autosampler temperature**: 5°

**System suitability**

**Sample**: *Standard solution*

[NOTE—See Table 1 for relative retention times.]

**Suitability requirements**

**Tailing factor**: NMT 2.0 for azithromycin and NMT 2.6 for *N-demethylazithromycin*

**Relative standard deviation**: NMT 10.0% for azithromycin N-oxide, desosaminylazithromycin, *N-demethylazithromycin*, and azithromycin



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each specified impurity in the portion of Azithromycin for Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times 100$$

$r_u$  = peak response of each specified impurity from the *Sample solution*

$r_s$  = peak response of each specified impurity from the *Standard solution*

$C_s$  = concentration of the relevant impurity USP RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of azithromycin in the *Sample solution* (mg/mL)

$P$  = potency of relevant USP RS (mg/mg)

**Acceptance criteria:** See Table 1. The reporting level for impurities is 0.05%.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azithromycin <i>N</i> -oxide <sup>a</sup>	0.17	1.0
Desosaminylazithromycin <sup>b</sup>	0.27	0.3
Erythromycin A oxime <sup>c,d</sup>	0.35	—
<i>N</i> -Demethylazithromycin <sup>e</sup>	0.50	1.0
Azaerythromycin A <sup>c,f</sup>	0.85	—
Azithromycin	1.00	—

<sup>a</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>b</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>c</sup> Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

<sup>d</sup> (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*)-6-[[3,4,6-Trideoxy-3-(dimethylamino)- $\beta$ -D-xyllo-hexopyranosyl]oxy]-14-ethyl-7,12,13-trihydroxy-4-[(2,6-dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethyloxacyclotetradecan-2-one.

<sup>e</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino- $\beta$ -D-xyllo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

<sup>f</sup> 9-Deoxy-9a-aza-9a-homoerythromycin A.

• **LIMIT OF AMINOAZITHROMYCIN, FORMAMIDO ANALOG, METHYLFORMAMIDO ANALOG, AND 3'-DE(DIMETHYLAMINO)-3'-OXOAZITHROMYCIN** (if present)

**Buffer:** 3.5 g/L of dibasic potassium phosphate in water

**Mobile phase:** Acetonitrile and *Buffer* (46:54). Adjust with 10 N potassium hydroxide to a pH of 11.0  $\pm$  0.1.

**Diluent:** Acetonitrile and water (46:54)

**Blank:** Use the *Diluent*.

**Standard stock solution:** 0.09 mg/mL of USP Desosaminylazithromycin RS, 0.21 mg/mL of USP *N*-

Demethylazithromycin RS, and 0.30 mg/mL of USP Azithromycin RS in acetonitrile

**Standard solution:** 0.0018 mg/mL of desosaminylazithromycin, 0.0042 mg/mL of *N*-demethylazithromycin, and 0.006 mg/mL of azithromycin in *Diluent*

**Sample solution:** Equivalent to 0.6 mg/mL of azithromycin from Azithromycin for Injection in *Diluent*. Reconstitute 3 vials individually, as directed in the labeling. Mix the contents of all the reconstituted vials. Dilute a portion of the mixture with *Diluent*. The *Sample solution* must be injected immediately after preparation.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Amperometric electrochemical detector

**Electrode:** Dual series glassy carbon electrodes

**Mode:** Oxidative screen mode

**Electrode 1:** +0.70  $\pm$  0.05V

**Electrode 2:** +0.82  $\pm$  0.05V

**Background current:** 95  $\pm$  25 nanoamperes

**Columns**

**Guard:** 4.6-mm  $\times$  1-cm; 5- $\mu$ m packing L67

**Analytical:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L67

**Temperature**

**Column:** 40°

**Autosampler:** 15°

**Flow rate:** 1 mL/min

**Injection size:** 25  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—See Table 2 for relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between desosaminylazithromycin and *N*-demethylazithromycin

**Tailing factor:** NMT 1.5 for azithromycin

**Relative standard deviation:** NMT 5% for azithromycin

**Analysis**

**Samples:** *Blank*, *Standard solution*, and *Sample solution*

Disregard any peaks corresponding to those obtained from the *Blank*.

Calculate the percentage of each impurity in the portion of Azithromycin for Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response of each impurity from the *Sample solution*

$r_s$  = peak response of azithromycin from the *Standard solution*

$C_s$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of azithromycin in the *Sample solution* (mg/mL)

$P$  = potency of USP Azithromycin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** See Table 2.



Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Erythromycin A iminoether <sup>a,b</sup>	0.20	—
3'-( <i>N,N</i> -Didemethyl)azithromycin (aminoazithromycin) <sup>c</sup> + 3'-( <i>N,N</i> -didemethyl)-3'- <i>N</i> -formylazithromycin (formamido analog) <sup>d</sup>	0.25	1.0
Azithromycin F <sup>a,e</sup>	0.30	—
Desosaminylazithromycin <sup>f,g</sup>	0.31	—
3'- <i>N</i> -Demethyl-3'- <i>N</i> -formylazithromycin (methylformamido analog) <sup>h</sup>	0.32	1.0
<i>N</i> -Demethylazithromycin <sup>i,j</sup>	0.35	—
Erythromycin A oxime <sup>a,l</sup>	0.42	—
Azaerythromycin A <sup>a,k</sup>	0.63	—
3'-De(dimethylamino)-3'-oxoazithromycin <sup>l</sup>	0.72	1.0
3'- <i>N</i> -Demethyl-3'- <i>N</i> -[(4-methylphenyl)sulfonyl]-azithromycin <sup>a,m</sup>	0.85	—
Azithromycin	1.00	—
Azithromycin B (3-Deoxy-azithromycin) <sup>a,n</sup>	1.64	—

<sup>a</sup> Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

<sup>b</sup> (3*R*,4*R*,5*S*,6*R*,9*R*,10*S*,11*S*,12*R*,13*S*,15*R*,Z)-12-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-6-ethyl-4,5-dihydroxy-10-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-3,5,9,11,13,15-hexamethyl-7,16-dioxo-2-azabicyclo[11.2.1]hexadec-1-en-8-one.

<sup>c</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>d</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>e</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(dimethylamino)-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>f</sup> These impurities are controlled using the Limit of Azithromycin *N*-Oxide Desosaminylazithromycin, and *N*-Demethylazithromycin test. They are listed here for information only.

<sup>g</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>h</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>i</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>j</sup> (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*,E)-6-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-14-ethyl-7,12,13-trihydroxy-4-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethyloxacyclotetradecan-2-one.

<sup>k</sup> 9-Deoxy-9a-aza-9a-homoerythromycin A.

<sup>l</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>m</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-acetamidophenyl)sulfonyl]-*N*-methylamino]-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>n</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>o</sup> Total impurities includes desosaminylazithromycin and *N*-demethylazithromycin.

Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any other unspecified impurity	—	0.2
Total impurities <sup>o</sup>	—	3.0

<sup>a</sup> Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

<sup>b</sup> (3*R*,4*R*,5*S*,6*R*,9*R*,10*S*,11*S*,12*R*,13*S*,15*R*,Z)-12-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-6-ethyl-4,5-dihydroxy-10-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-3,5,9,11,13,15-hexamethyl-7,16-dioxo-2-azabicyclo[11.2.1]hexadec-1-en-8-one.

<sup>c</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>d</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>e</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>f</sup> These impurities are controlled using the Limit of Azithromycin *N*-Oxide Desosaminylazithromycin, and *N*-Demethylazithromycin test. They are listed here for information only.

<sup>g</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>h</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>i</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>j</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethyloxacyclotetradecan-2-one.

<sup>k</sup> 9-Deoxy-9a-aza-9a-homoerythromycin A.

<sup>l</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>m</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-acetamidophenyl)sulfonyl]-*N*-methylamino]-3,4,6-trideoxy-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>n</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl]oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>o</sup> Total impurities includes desosaminylazithromycin and *N*-demethylazithromycin.

## SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.7 USP Endotoxin Units/mg of azithromycin.
- **STERILITY TESTS (71):** It meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined, Membrane Filtration*.
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements.
- **PH (791):** 6.4–6.8, determined in a solution constituted as directed in the labeling.
- **WATER DETERMINATION, Method I (921):** NMT 2.0%.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products (1)*.

## ADDITIONAL REQUIREMENTS

### Change to read:

- **PACKAGING AND STORAGE:** Preserve as described under *Packaging and Storage Requirements (659), Injection*



Packaging, Packaging for constitution (CN 1-May-2017). Store at controlled room temperature.

- **LABELING:** It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*.

- **USP REFERENCE STANDARDS (11)**

USP Azaerythromycin A RS

9-Deoxy-9a-aza-9a-homoerythromycin A.

$C_{37}H_{70}N_2O_{12}$  734.96

USP Azithromycin RS

USP Azithromycin N-Oxide RS

(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylaziridinyl)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

$C_{38}H_{72}N_2O_{13}$  764.98

USP N-Demethylazithromycin RS

(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[(2,6-Dideoxy-3-C-methyl-3-O-methyl- $\alpha$ -L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

$C_{37}H_{70}N_2O_{12}$  734.96

USP Desosaminylazithromycin RS

(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

$C_{30}H_{58}N_2O_9$  590.79

USP Endotoxin RS

## Azithromycin for Oral Suspension

### DEFINITION

Azithromycin for Oral Suspension is a dry mixture of Azithromycin and one or more buffers, sweeteners, diluents, anticaking agents, and flavors. It contains NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ).

### IDENTIFICATION

- **A.** The retention time of the azithromycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

[NOTE—Use water that has a resistivity of NLT 18 Mohm-cm.]

**Mobile phase:** Dissolve 5.8 g of monobasic potassium phosphate in 2130 mL of water, and add 870 mL of acetonitrile. Adjust with about 6 mL of 10 N potassium hydroxide to a pH of  $11.0 \pm 0.1$ , and pass through a suitable filter.

**Diluent:** Dissolve 2.2 g of monobasic potassium phosphate in 1590 mL of water, and add 600 mL of isopropyl alcohol, 480 mL of alcohol, and 330 mL of acetonitrile. Adjust with 10 N potassium hydroxide to a pH of  $8.4 \pm 0.1$ , and shake by mechanical means for 30 min.

**Standard stock solution:** 0.165 mg/mL of USP Azithromycin RS in acetonitrile. Swirl, and sonicate as necessary.

**Standard solution:** 3.3  $\mu$ g/mL of USP Azithromycin RS from the *Standard stock solution* in *Mobile phase*

**System suitability stock solution:** 0.16 mg/mL of USP Azaerythromycin A RS in acetonitrile and *Mobile phase* (1:9). Dissolve first in acetonitrile using 10% of the final volume. Swirl, and sonicate to dissolve. Dilute with *Mobile phase* to volume.

**System suitability solution:** 3.2  $\mu$ g/mL of azaerythromycin A from the *System suitability stock solution* and 3.3  $\mu$ g/mL of azithromycin from the *Standard stock solution* in *Mobile phase*

**Sample stock solution 1** (where packaged in a single-unit container): 2 mg/mL of azithromycin from Azithromycin for Oral Suspension in *Diluent*. Transfer the contents of a container of Azithromycin for Oral Suspension to a suitable volumetric flask. Add *Diluent* equal to 70% of the volume of the flask, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume. Transfer 40 mL of this suspension to a stoppered centrifuge tube, and centrifuge for 20 min. Use the supernatant to prepare *Sample solution 1*.

**Sample stock solution 2** (where packaged in a multiple-unit container): 0.4 mg/mL of azithromycin from Azithromycin for Oral Suspension in *Diluent*. Constitute Azithromycin for Oral Suspension as directed in the labeling. Transfer a suitable aliquot of the suspension so obtained, freshly mixed and free from air bubbles, to a suitable volumetric flask to obtain a final concentration of 0.4 mg/mL. Add *Diluent* equal to 70% of the final volume, shake by mechanical means for 30 min, and dilute with *Diluent* to volume. Transfer 40 mL of the suspension so obtained to a stoppered centrifuge tube, and centrifuge for 20 min. Use the supernatant to prepare *Sample solution 2*.

**Sample solution 1:** 3.2  $\mu$ g/mL of azithromycin from *Sample stock solution 1* in *Mobile phase*

**Sample solution 2:** 4  $\mu$ g/mL of azithromycin from *Sample stock solution 2* in *Mobile phase*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Amperometric electrochemical detector

**Electrode:** Dual glassy carbon

**Mode:** Oxidative screen mode

**Electrode 1:**  $+0.70 \pm 0.05$  V

**Electrode 2:**  $+0.82 \pm 0.05$  V

**Background current:**  $85 \pm 15$  nanoamperes

### Columns

**Guard:** 4.6-mm  $\times$  5-cm; 5- $\mu$ m packing L29

**Analytical:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L29 or 3- $\mu$ m packing L49 without the *Guard column*

**Flow rate:** 1.5 mL/min

**Injection volume:** 50  $\mu$ L

### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for azaerythromycin A and azithromycin with the L29 column are 0.7 and 1.0, respectively; the relative retention times for azaerythromycin A and azithromycin with the L49 column are 0.8 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*

**Column efficiency:** NLT 1000 theoretical plates, *Standard solution*

**Tailing factor:** 0.9–1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution*, *Sample solution 1*, or *Sample solution 2*

**Where packaged in a single-unit container**

Calculate the percentage of the labeled amount of azithromycin ( $C_{38}H_{72}N_2O_{12}$ ) in the portion of Azithromycin for Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response from *Sample solution 1*

$r_s$  = peak response from the *Standard solution*



- $C_s$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of azithromycin in *Sample solution 1* (mg/mL)  
 $P$  = potency of azithromycin in USP Azithromycin RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

Where packaged in a multiple-unit container

Calculate the percentage of the labeled amount of azithromycin ( $\text{C}_{38}\text{H}_{72}\text{N}_2\text{O}_{12}$ ) in the portion of Azithromycin for Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

- $r_u$  = peak response from *Sample solution 2*  
 $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of azithromycin in *Sample solution 2* (mg/mL)  
 $P$  = potency of azithromycin in USP Azithromycin RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
 Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

- **DELIVERABLE VOLUME** (698): Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS** (905)  
For single-unit containers  
Acceptance criteria: Meets the requirements

#### SPECIFIC TESTS

- **pH** (791)  
For a solid packaged in single-unit containers:  
9.0–11.0, in the suspension constituted as directed in the labeling  
For a solid packaged in multiple-unit containers:  
8.5–11.0, in the suspension constituted as directed in the labeling

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Azaerythromycin A RS  
9-Deoxy-9a-aza-9a-homoerythromycin A;  
6-Demethylazithromycin,  
 $\text{C}_{37}\text{H}_{70}\text{N}_2\text{O}_{12}$  734.96  
USP Azithromycin RS

## Azithromycin Tablets

#### DEFINITION

Azithromycin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ( $\text{C}_{38}\text{H}_{72}\text{N}_2\text{O}_{12}$ ).

#### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

**Buffer:** Dissolve 4.6 g of monobasic potassium phosphate anhydrous in 900 mL of water. Adjust with 1 N sodium hydroxide to a pH of 7.5, and dilute with water to 1 L.

**Mobile phase:** Acetonitrile and *Buffer* (65:35)

**Standard solution:** 1 mg/mL of USP Azithromycin RS in *Mobile phase*. Sonicate and shake as needed to dissolve.

**Sample solution:** Nominally 1 mg/mL of azithromycin in *Mobile phase* from NLT 20 Tablets, finely powdered. Sonicate and shake as needed to dissolve.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu\text{m}$  packing L1

**Column temperature:** 50°

**Flow rate:** 2 mL/min

**Injection volume:** 100  $\mu\text{L}$

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of azithromycin ( $\text{C}_{38}\text{H}_{72}\text{N}_2\text{O}_{12}$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

- $r_u$  = peak response of azithromycin from the *Sample solution*  
 $r_s$  = peak response of azithromycin from the *Standard solution*  
 $C_s$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of azithromycin in the *Sample solution* (mg/mL)  
 $P$  = potency of USP Azithromycin RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
 Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

##### DISSOLUTION (711)

**Medium:** pH 6.0 phosphate buffer; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Solution A:** 4.4 mg/mL of dibasic potassium phosphate and 0.5 mg/mL of sodium 1-octanesulfonate, adjusted with phosphoric acid to a pH of  $8.20 \pm 0.05$

**Mobile phase:** Acetonitrile, methanol, and *Solution A* (9:3:8)

**Diluent:** 17.5 mg/mL of dibasic potassium phosphate. Adjust with phosphoric acid to a pH of  $8.00 \pm 0.05$ . Prepare a mixture of this solution and acetonitrile (80:20).

**Standard stock solution:** Dissolve USP Azithromycin RS in *Medium* to obtain a solution having a known concentration of about  $(L/1000)$  mg/mL, where  $L$  is the Tablet label claim in mg.

**Standard solution:** Dilute the *Standard stock solution* with *Diluent* to obtain a solution having a known concentration of about  $(L/2000)$  mg/mL, where  $L$  is the Tablet label claim in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu\text{m}$  pore size. Dilute a portion of the filtrate with *Diluent* to obtain a solution having a theoretical concentration of about  $(L/2000)$  mg/mL, where  $L$  is the Tablet label claim in mg, assuming complete dissolution.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 210 nm  
 Column: 4.6-mm × 15-cm; 5-μm packing L1  
 Column temperature: 50°  
 Flow rate: 1.5 mL/min  
 Injection volume: 50 μL  
 System suitability  
 Sample: *Standard solution*  
 Suitability requirements  
 Tailing factor: NMT 2.0  
 Relative standard deviation: NMT 2.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of azithromycin (C<sub>38</sub>H<sub>72</sub>N<sub>2</sub>O<sub>12</sub>) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response of azithromycin from the *Sample solution*

$r_S$  = peak response of azithromycin from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of azithromycin (C<sub>38</sub>H<sub>72</sub>N<sub>2</sub>O<sub>12</sub>) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

Protect all solutions containing azithromycin from light. Refrigerate the *Standard solution* and the *Sample solution* after preparation and during analysis, using a refrigerated autosampler set at 4°. The solutions must be analyzed within 24 h of preparation.

**Solution A:** Water and ammonium hydroxide (2000:1.2). The pH of this solution is about 10.5.

**Solution B:** Acetonitrile, methanol, and ammonium hydroxide (1800:200:1.2)

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	54	46
20	54	46
35	10	90
35.1	54	46
40	54	46

**Buffer:** 1.7 g/L of monobasic ammonium phosphate in water. Adjust with ammonium hydroxide to a pH of 10.

**Diluent A:** Methanol, acetonitrile, and *Buffer* (350:300:350)

**Diluent B:** Methanol and *Buffer* (1:1)

**System suitability stock solution:** 0.1 mg/mL each of USP Desosaminylazithromycin RS and USP Azithromycin Related Compound F RS in acetonitrile

**System suitability solution:** 0.028 mg/mL each of USP Desosaminylazithromycin RS and USP Azithromycin Related Compound F RS from *System suitability stock solution* in *Diluent A*

**Standard stock solution:** 0.4 mg/mL of USP Azithromycin RS in acetonitrile. Sonicate and shake as needed to dissolve.

**Standard solution:** 0.02 mg/mL of azithromycin from *Standard stock solution* in *Diluent A*

**Sensitivity solution:** 0.004 mg/mL of azithromycin from *Standard solution* in *Diluent A*

**Sample stock solution:** Nominally 14.3 mg/mL of azithromycin prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer nominally 1430 mg of azithromycin to a 100-mL volumetric flask. Add 75 mL of acetonitrile, and sonicate for NLT 15 min. Shake by mechanical means for NLT 15 min. Allow the solution to equilibrate to room temperature, dilute with acetonitrile to volume, and mix.

**Sample solution:** Nominally 4 mg/mL of azithromycin prepared as follows. Centrifuge an aliquot of the *Sample stock solution* for NLT 15 min. Transfer 7.0 mL of the supernatant to a 25-mL volumetric flask, and dilute with *Diluent B* to volume.

**Blank:** *Diluent A*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Column temperature: 50°

Flow rate: 1.2 mL/min

Autosampler temperature: 4°

Injection volume: 100 μL

#### System suitability

Samples: *System suitability solution*, *Standard solution*, and *Sensitivity solution*

#### Suitability requirements

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Resolution:** NLT 1.0 between desosaminylazithromycin and azithromycin related compound F, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

Samples: *Sample solution* and *Blank*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F_1 \times (1/F_2) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of azithromycin from the *Standard solution*

$C_S$  = concentration of USP Azithromycin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of azithromycin in the *Sample solution* (mg/mL)

$P$  = potency of USP Azithromycin RS (μg/mg)

$F_1$  = conversion factor, 0.001 mg/μg

$F_2$  = relative response factor (see Table 2)

**Acceptance criteria:** See Table 2. The reporting level for impurities is 0.1%. Disregard any peaks in the *Sample solution* that correspond to peaks in the *Blank*.



Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Azithromycin <i>N</i> -oxide <sup>a</sup>	0.20	0.42	1.0
3'-( <i>N,N</i> -Didemethyl)-3'- <i>N</i> -formylazithromycin <sup>b</sup>	0.29	1.7	1.0
3'-( <i>N,N</i> -Didemethyl)azithromycin(ami-noazithromycin) <sup>c</sup>	0.34	0.49	0.5
Azithromycin related compound F <sup>d</sup>	0.42	4.3	1.0
Desosaminylazithromycin <sup>e</sup>	0.46	1.1	0.5
<i>N</i> -Demethylazithromycin <sup>f</sup>	0.50	0.54	0.7
3'-De(dimethylamino)-3'-oxoazithromycin <sup>g</sup>	0.87	1.4	1.0
Azaerythromycin A <sup>h,i</sup>	0.94	—	—
Azithromycin	1.0	—	—
2-Desethyl-2-propylazithromycin <sup>h,i</sup>	1.10	—	—
3'- <i>N</i> -Demethyl-3'- <i>N</i> -[(4-methylphenyl)sulfonyl]azithromycin <sup>h,k</sup>	1.11	—	—

<sup>a</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>b</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>c</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>d</sup> 3'-(*N*-Demethyl)-3'-*N*-formylazithromycin; (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>e</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>f</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>g</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>h</sup> Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only. The unspecified impurities and total impurities limits do not include these impurities.

<sup>i</sup> 9-Deoxy-9a-aza-9a-homoerythromycin A.

<sup>j</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-propyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one dihydrate.

<sup>k</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-methylphenyl)sulfonyl]-*N*-methylamino]-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>l</sup> (2*R*,3*S*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
3-Deoxyazithromycin (azithromycin B) <sup>h,i</sup>	1.14	—	—
Any individual unspecified impurity <sup>h</sup>	—	1.0	0.2
Total impurities <sup>h</sup>	—	—	5.0

<sup>a</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>b</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>c</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>d</sup> 3'-(*N*-Demethyl)-3'-*N*-formylazithromycin; (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>e</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>f</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>g</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>h</sup> Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only. The unspecified impurities and total impurities limits do not include these impurities.

<sup>i</sup> 9-Deoxy-9a-aza-9a-homoerythromycin A.

<sup>j</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-propyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one dihydrate.

<sup>k</sup> (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-methylphenyl)sulfonyl]-*N*-methylamino]-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

<sup>l</sup> (2*R*,3*S*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

## • USP REFERENCE STANDARDS (11)

USP Azithromycin RS

USP Azithromycin Related Compound F RS

3'-(*N*-Demethyl)-3'-*N*-formylazithromycin;  
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl- $\alpha$ -*L*-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

C<sub>38</sub>H<sub>70</sub>N<sub>2</sub>O<sub>13</sub> 762.97

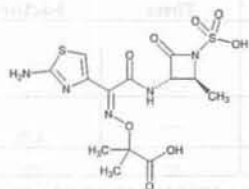
USP Desosaminylazithromycin RS

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino- $\beta$ -D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclotetradecan-15-one.

C<sub>30</sub>H<sub>58</sub>N<sub>2</sub>O<sub>9</sub> 590.79



## Aztreonam



$C_{13}H_{17}N_5O_8S_2$  435.43

Propanoic acid, 2-[[[1-(2-amino-4-thiazolyl)-2-[(2-methyl-4-oxo-1-sulfo-3-azetidinyl)amino]-2-oxoethylidene]amino]oxy]-2-methyl-, [2S-[2 $\alpha$ ,3 $\beta$ (Z)]]-; (Z)-2-[[[1-(2-Amino-4-thiazolyl)-[(2S,3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl]methylene]amino]oxy]-2-methylpropionic acid [78110-38-0].

### DEFINITION

Aztreonam, which may be anhydrous or hydrated, contains NLT 92.0% and NMT 105.0% of  $C_{13}H_{17}N_5O_8S_2$ , calculated on the anhydrous and solvent-free basis.

### IDENTIFICATION

- INFRARED ABSORPTION (197K):** If a difference appears in the IR spectra of the analyte and the standard, dissolve equal portions of the test specimen and the reference standard in equal volumes of methanol. [NOTE—To achieve a complete dissolution, it is suggested to use about 25 mL of methanol for each 50 mg of material, and stir the mixture for 40 min at room temperature.] Evaporate the solutions to dryness under vacuum, and dry at 40° for 4 h under vacuum. Perform the test on the residues.

### ASSAY

#### PROCEDURE

[NOTE—Store the *System suitability solution*, *Standard solution*, and *Sample solution* at 5°, and protect from light to prevent isomerization of aztreonam Z-isomer to aztreonam E-isomer.]

**Buffer:** 6.8 mg/mL of monobasic potassium phosphate in water. Adjust with 1 M phosphoric acid to a pH of 3.0.

**Mobile phase:** Methanol and *Buffer* (1:4)

**System suitability solution:** 1 mg/mL of USP Aztreonam RS and 1 mg/mL of USP Aztreonam E-Isomer RS in *Mobile phase*

**Standard solution:** 1 mg/mL of USP Aztreonam RS in *Mobile phase*

**Sample solution:** 1 mg/mL of Aztreonam in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for aztreonam and aztreonam E-isomer are 1.0 and 1.8, respectively.]

### Suitability requirements

**Resolution:** NLT 2.0 between aztreonam and aztreonam E-isomer, *System suitability solution*

**Tailing factor:** NMT 2 for aztreonam, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ) in the portion of Aztreonam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aztreonam in the *Sample solution* (mg/mL)

$P$  = potency of USP Aztreonam RS ( $\mu$ g/mg)

$F$  = unit conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 92.0%–105.0% on the anhydrous and solvent-free basis

### IMPURITIES

#### Inorganic Impurities

- RESIDUE ON IGNITION (281):** NMT 0.1%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

#### Delete the following:

- HEAVY METALS, Method II (231):** NMT 30 ppm (Official 1-Jan-2018)

### Organic Impurities

#### PROCEDURE

[NOTE—Store the *System suitability solution*, *Standard solution*, and *Sample solution* at 5°, and protect from light to prevent isomerization of aztreonam Z-isomer to aztreonam E-isomer.]

**Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Aztreonam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of aztreonam from the *Standard solution*

$C_S$  = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Aztreonam in the *Sample solution* (mg/mL)

$P$  = potency of USP Aztreonam RS ( $\mu$ g/mg)

$F$  = unit conversion factor, 0.001 mg/ $\mu$ g

#### Acceptance criteria

**Individual impurities:** See Table 1.



Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Open-ring aztreonam <sup>a</sup> and open-ring desulfated aztreonam <sup>b,c</sup>	0.55	1.0
Aztreonam (Z-isomer)	1.0	—
Desulfated aztreonam <sup>d</sup>	1.6	1.5
Aztreonam E-isomer <sup>e</sup>	1.8	0.5
Aztreonam ethyl ester <sup>f</sup>	3.9	1.5
Any individual unspecified impurity	—	0.1
Total impurities	—	3.0

<sup>a</sup> (2S,3S)-2-((Z)-2-[2-Aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino]acetamido)-3-(sulfoamino)butanoic acid.

<sup>b</sup> (2S,3S)-3-Amino-2-((Z)-2-[2-aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino]acetamido)butanoic acid.

<sup>c</sup> Open-ring aztreonam and open-ring desulfated aztreonam coelute. The limit is for the sum of these two impurities.

<sup>d</sup> (Z)-2-(((2-Amino-4-thiazolyl))((2S,3S)-2-methyl-4-oxo-3-azetidinyl)carbamoyl)methyleneamino)oxy)-2-methylpropionic acid.

<sup>e</sup> (E)-2-(((2-Amino-4-thiazolyl))((2S,3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl)carbamoyl)methyleneamino)oxy)-2-methylpropionic acid.

<sup>f</sup> Ethyl (Z)-2-(((2-amino-4-thiazolyl))((2S,3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl)carbamoyl)methyleneamino)oxy)-2-methylpropionate.

### SPECIFIC TESTS

- **STERILITY TESTS (71):** Where the label states that Aztreonam is sterile, it meets the requirements for *Test for Sterility of the Product to Be Examined—Membrane Filtration*, using *Fluid A*, to which 23.4 g of sterile arginine has been added to each 1000 mL.
- **WATER DETERMINATION, Method I (921):** NMT 2.0%; if labeled as the hydrated form: 12.0%–18.0%. [NOTE—The term hydrated form refers to the α-form of Aztreonam, which is not a stoichiometric hydrate.]
- **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that aztreonam is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.17 USP Endotoxin Unit/mg of aztreonam.
- **LIMIT OF ALCOHOL**

[NOTE—This test is to be performed if alcohol is used while manufacturing Aztreonam.]

**Standard solution:** 0.004 mL/mL of alcohol from USP Alcohol Determination—Alcohol RS and 0.004 mL/mL of acetonitrile from USP Alcohol Determination—Acetonitrile RS in dimethylformamide. [NOTE—The *Standard solution* contains 0.4% alcohol and 0.4% acetonitrile.]

**Sample solution:** 80 mg/mL of Aztreonam and 0.004 mL/mL of acetonitrile in dimethylformamide. [NOTE—Dissolve Aztreonam in dimethylformamide using 20% of the final volume. Add a suitable aliquot of USP Alcohol Determination—Acetonitrile RS, and dilute with dimethylformamide to volume. The concentration of acetonitrile in the *Sample solution* is 0.4%.]

#### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m; phase G43

Film thickness: 3.0 μm

Temperature

Injector: 210°

Detector: 280°

Column: See Table 2.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	0	50	5

Table 2 (Continued)

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
50	10	200	4

Carrier gas: He

Linear velocity: 35 cm/s

Injection mode: Split

Split ratio: 5:1

Injection size: 0.5 μL

System suitability

[NOTE—The relative retention times for alcohol and acetonitrile are 1.0 and 1.3, respectively.]

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between alcohol and acetonitrile

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of alcohol in the portion of Aztreonam taken:

$$\text{Result} = (R_U/R_S) \times (C_S \times D/C_U) \times F \times 100$$

$R_U$  = peak response ratio of alcohol to acetonitrile from the *Sample solution*

$R_S$  = peak response ratio of alcohol to acetonitrile from the *Standard solution*

$C_S$  = concentration of alcohol in the *Standard solution* (mL/mL)

$D$  = density of alcohol (g/mL)

$C_U$  = concentration of Aztreonam in the *Sample solution* (mg/mL)

$F$  = unit conversion factor, 1000 mg/g

Acceptance criteria: NMT 4%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Where it is the hydrated form, the label so indicates.
- **USP REFERENCE STANDARDS (11)**
  - USP Alcohol Determination—Acetonitrile RS
  - $C_2H_3N$  41.05
  - USP Alcohol Determination—Alcohol RS
  - $C_2H_5OH$  46.07
  - USP Aztreonam RS
  - Propanoic acid, 2-[[[1-(2-amino-4-thiazolyl)-2-[(2-methyl-4-oxo-1-sulfo-3-azetidinyl)amino]-2-oxoethylidene]amino]oxy]-2-methyl-, [2S-[2α,3β(Z)]]-
  - $C_{13}H_{17}N_5O_8S_2$  435.43
  - USP Aztreonam E-Isomer RS
  - (E)-2-[[[1-(2-Amino-4-thiazolyl)]((2S,3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl)carbamoyl)methyleneamino]oxy)-2-methylpropionic acid.
  - $C_{13}H_{17}N_5O_8S_2$  435.43
  - USP Endotoxin RS

## Aztreonam Injection

### DEFINITION

Aztreonam Injection is a sterile solution of Aztreonam and Arginine and a suitable osmolality-adjusting substance in Water for Injection. It contains NLT 90.0% and NMT 120.0% of the labeled amount of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ).



**IDENTIFICATION**

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 1.15 g/L of monobasic ammonium phosphate in water. Before final dilution, adjust with phosphoric acid to a pH of  $2.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (75:25)

**System suitability solution:** 0.2 mg/mL each of USP Aztreonam RS and USP Open Ring Aztreonam RS in *Mobile phase*

**Standard solution:** 0.2 mg/mL each of USP Aztreonam RS and USP L-Arginine RS in *Mobile phase*

**Sample solution:** Nominally 0.2 mg/mL of aztreonam from Injection in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 206 nm

**Column:** 4-mm  $\times$  25-cm; 5- to 10- $\mu$ m packing L20

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for aztreonam and open ring aztreonam are 0.8 and 1.0, respectively. The relative retention times for aztreonam and arginine are 0.3 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between aztreonam and open ring aztreonam

**Tailing factor:** NMT 2.0 for the aztreonam peak

**Relative standard deviation:** NMT 2.0% for the aztreonam peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of aztreonam from the *Sample solution*

$r_S$  = peak response of aztreonam from the *Standard solution*

$C_S$  = concentration of USP Aztreonam RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aztreonam in the *Sample solution* (mg/mL)

$P$  = potency of aztreonam in USP Aztreonam RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–120.0%

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.25 USP Endotoxin Unit/mg of aztreonam
- **STERILITY TESTS** (71): It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **pH** (791): 4.5–7.5
- **PARTICULATE MATTER IN INJECTIONS** (788): It meets the requirements for small-volume injections.

**ADDITIONAL REQUIREMENTS****Change to read:**

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*,

*Packaging for constitution* (CN 1-May-2017). Maintain in the frozen state.

- **LABELING:** It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that the Injection is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

• **USP REFERENCE STANDARDS** (11)

USP L-Arginine RS

USP Aztreonam RS

USP Endotoxin RS

USP Open Ring Aztreonam RS

(2S,3S)-2-[(Z)-2-[2-Aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino]acetamido]-3-(sulfoamino)butanoic acid.

$C_{13}H_{19}N_5O_8S_2$  453.45

**Aztreonam for Injection****DEFINITION**

Aztreonam for Injection is a dry mixture of sterile Aztreonam and Arginine. It contains NLT 90.0% and NMT 105.0% of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ), calculated on the anhydrous and arginine-free basis. Each container contains NLT 90.0% and NMT 120.0% of the labeled amount of aztreonam ( $C_{13}H_{17}N_5O_8S_2$ ).

**IDENTIFICATION**

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 1.15 g/L of monobasic ammonium phosphate in water. Before final dilution, adjust with phosphoric acid to a pH of  $2.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (75:25)

**System suitability solution:** 0.2 mg/mL each of USP Aztreonam RS and USP Open Ring Aztreonam RS in *Mobile phase*

**Standard solution:** 0.2 mg/mL each of USP Aztreonam RS and USP L-Arginine RS in *Mobile phase*

**Sample solution 1:** Nominally 0.2 mg/mL of aztreonam in *Mobile phase* from Aztreonam for Injection prepared as follows. Weigh one container of Aztreonam for Injection, transfer the contents to a suitable container, and dilute with *Mobile phase* to the appropriate volume. Weigh the empty container, and calculate the weight, in mg, of Aztreonam for Injection used.

**Sample solution 2:** Nominally 0.2 mg/mL of aztreonam from Aztreonam for Injection constituted as directed below and diluted with *Mobile phase*.

Where the vial has a capacity of less than 100 mL, constitute with water using the volume of solvent specified in the labeling.

Where the vial capacity is  $\geq 100$  mL, constitute with 10 mL of water and dilute the entire withdrawable contents of the container with *Mobile phase* to obtain the final concentration.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 206 nm

**Column:** 4-mm  $\times$  25-cm; 5- to 10- $\mu$ m packing L20

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for aztreonam and open ring aztreonam are about 0.8 and 1.0, respec-



tively. The relative retention times for aztreonam and arginine are 0.3 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between aztreonam and open ring aztreonam

**Tailing factor:** NMT 2.0 for the aztreonam peak

**Relative standard deviation:** NMT 2.0% for the aztreonam peak

#### Analysis

**Samples:** Standard solution, Sample solution 1, and Sample solution 2

Calculate the percentage of the labeled amount of aztreonam ( $C_{13}H_{17}N_5O_6S_2$ ) in the portion of Aztreonam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response for aztreonam from Sample solution 1

$r_S$  = peak response for aztreonam from the Standard solution

$C_S$  = concentration of USP Aztreonam RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of Aztreonam for Injection in Sample solution 1 (mg/mL), corrected for water and arginine content (see Content of Arginine)

$P$  = potency of aztreonam in USP Aztreonam RS ( $\mu\text{g}/\text{mg}$ )

$F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

**Acceptance criteria:** 90.0%–105.0% on the anhydrous and arginine-free basis

Calculate the percentage of the labeled amount of aztreonam ( $C_{13}H_{17}N_5O_6S_2$ ) in each container of Aztreonam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response for aztreonam from Sample solution 2

$r_S$  = peak response for aztreonam from the Standard solution

$C_S$  = concentration of USP Aztreonam RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of aztreonam in Sample solution 2 (mg/mL)

$P$  = potency of aztreonam in USP Aztreonam RS ( $\mu\text{g}/\text{mg}$ )

$F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

**Acceptance criteria:** 90.0%–120.0%

#### OTHER COMPONENTS

##### • CONTENT OF ARGININE

Use the result of this test to calculate, on the anhydrous and arginine-free basis, the Assay result from Sample solution 1, obtained as directed in the Assay.

**Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution 1, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

#### Analysis

**Sample:** Sample solution 1

Calculate the percentage of arginine ( $C_6H_{14}N_4O_2$ ) in the portion of Aztreonam for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for arginine from Sample solution 1

$r_S$  = peak response for arginine from the Standard solution

$C_S$  = concentration of USP L-Arginine RS in the Standard solution (mg/mL)

$C_U$  = concentration of Aztreonam for Injection in Sample solution 1 (mg/mL)

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

#### SPECIFIC TESTS

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products (1)*, *Specific Tests, Completeness and clarity of solutions*.
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.17 USP Endotoxin Unit/mg of aztreonam.
- **STERILITY TESTS (71):** It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **pH (791)**  
Sample solution: 100 mg/mL of aztreonam  
Acceptance criteria: 4.5–7.5
- **WATER DETERMINATION, Method I (921):** NMT 2.0%
- **PARTICULATE MATTER IN INJECTIONS (788):** It meets the requirements for small-volume injections.
- **OTHER REQUIREMENTS:** It meets the requirements for *Labeling (7)*, *Labels and Labeling for Injectable Products*.

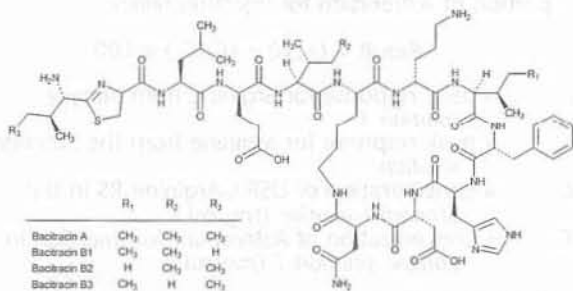
#### ADDITIONAL REQUIREMENTS

##### Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements (659)*, *Injection Packaging, Packaging for constitution*. (CN 1-May-2017)
- **USP REFERENCE STANDARDS (11)**  
USP L-Arginine RS  
USP Aztreonam RS  
USP Endotoxin RS  
USP Open Ring Aztreonam RS  
(2S,3S)-2-[(Z)-2-[2-Aminothiazol-4-yl]-2-[2-carboxypropan-2-yloxyimino]acetamido]-3-(sulfoamino)butanoic acid.  
 $C_{13}H_{19}N_5O_9S_2$  453.45



## Bacitracin



Bacitracin [1405-87-4].

### DEFINITION

Bacitracin is a mixture of polypeptides produced by the growth of an organism of the *licheniformis* group of *Bacillus subtilis* (Fam. Bacillaceae), the main components being bacitracins A, B1, B2, and B3. It has a potency of NLT 65 Bacitracin Units/mg, calculated on the dried basis.

### IDENTIFICATION

- **A.** Meets the requirements of the test for *Composition of Bacitracin*

- **B.**

Sample: 0.2 g

Analysis: Ignite the *Sample*. Allow to cool. Dissolve the residue in 0.1 mL of dilute hydrochloric acid. Add 5 mL of water and 0.2 mL of sodium hydroxide.

Acceptance criteria: No white precipitate is formed.

### ASSAY

#### • PROCEDURE

(See *Antibiotics—Microbial Assays* (81).)

Analysis: Proceed as directed in the chapter.

Acceptance criteria: NLT 65 Bacitracin Units/mg on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.5%

### SPECIFIC TESTS

#### • COMPOSITION OF BACITRACIN

Diluent: 40 g/L of edetate disodium in water adjusted with 8 N sodium hydroxide to a pH of 7.0

Solution A: 34.8 g/L of dibasic potassium phosphate in water

Solution B: 27.2 g/L of monobasic potassium phosphate in water

Solution C: *Solution B* and *Solution A* (9:2). The pH of the mixture is about 6.

Solution D: 0.1 mM edetate disodium in a mixture of *Solution C* and water (1:3)

Solution E: Methanol and acetonitrile (27:2)

Mobile phase: *Solution E* and *Solution D* (63:37)

System suitability solution: 2 mg/mL of USP Bacitracin Zinc RS in *Diluent*

Reporting threshold solution: 0.01 mg/mL of USP Bacitracin Zinc RS from *System suitability solution* in water

Peak identification solution: 2 mg/mL of USP Bacitracin Zinc RS in *Diluent*. Heat in a boiling water bath for 30 min, and cool to room temperature.

Sample solution: 2 mg/mL of Bacitracin in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm and 300 nm. Quantitative analysis is performed at 254 nm; 300 nm is only used to identify the location of bacitracin F.

Column: 4.6-mm × 25-cm; end-capped 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 100 μL

#### System suitability

Samples: *System suitability solution* and *Peak identification solution*

Analyze the *Peak identification solution* at 300 nm. Identify bacitracin F, a known impurity, using the relative retention time provided in *Table 1*. Analyze the *System suitability solution* at 254 nm. Identify the peaks of the most active components of bacitracin (bacitracins A, B1, B2, and B3), early eluting peptides (those eluting before the bacitracin B1 peak), and the impurity (bacitracin F) using the relative retention time values in *Table 1*.

Table 1

Name	Nature of Component	Relative Retention Time
Bacitracin C1	Early eluting peptides	0.5
Bacitracin C2		0.6
Bacitracin C3		0.6
Bacitracin B1	Active bacitracin	0.7
Bacitracin B2		0.7
Bacitracin B3		0.8
Bacitracin A		1.0
Bacitracin F	Impurity	2.4

#### Suitability requirements

Peak-to-valley ratio: NLT 1.2

The *Peak-to-valley ratio* is calculated as follows:

$$\text{Result} = H_p/H_v$$

$H_p$  = height above the baseline of the peak due to bacitracin B1

$H_v$  = height above the baseline of the lowest point of the curve separating the bacitracin B1 peak from the bacitracin B2 peak

#### Analysis

Samples: *Diluent*, *Reporting threshold solution*, and *Sample solution*

Quantitative analysis is performed at 254 nm.

#### Content of bacitracin A

Calculate the percentage of bacitracin A in the portion of Bacitracin taken:

$$\text{Result} = (r_A/r_T) \times 100$$

$r_A$  = peak area of bacitracin A from the *Sample solution*

$r_T$  = sum of all peak areas above the reporting threshold from the *Sample solution*

#### Content of active bacitracin

Calculate the percentage of active bacitracin (bacitracin A, B1, B2, and B3) in the portion of Bacitracin taken:

$$\text{Result} = [(r_A + r_{B1} + r_{B2} + r_{B3})/r_T] \times 100$$

$r_A$  = peak area of bacitracin A from the *Sample solution*

$r_{B1}$  = peak area of bacitracin B1 from the *Sample solution*

$r_{B2}$  = peak area of bacitracin B2 from the *Sample solution*

$r_{B3}$  = peak area of bacitracin B3 from the *Sample solution*

$r_T$  = sum of all peak areas above the reporting threshold from the *Sample solution*



**Limit of early eluting peptides**

Calculate the percentage of early eluting peptides (peaks eluting before bacitracin B1) in the portion of Bacitracin taken:

$$\text{Result} = (r_p/r_t) \times 100$$

- $r_p$  = sum of peak areas for all peaks before bacitracin B1 from the *Sample solution*  
 $r_t$  = sum of all peak areas above the reporting threshold from the *Sample solution*

**Limit of bacitracin F**

Calculate the percentage of bacitracin F in the portion of Bacitracin taken:

$$\text{Result} = (r_F/r_A) \times 100$$

- $r_F$  = peak area for bacitracin F from the *Sample solution*  
 $r_A$  = peak area for bacitracin A from the *Sample solution*

**Acceptance criteria:** See Table 2. Disregard any peaks from the *Sample solution* that are observed in the *Diluent* chromatogram. Disregard any peaks from the *Sample solution* having a peak area less than bacitracin A in the *Reporting threshold solution*.

**Table 2**

	Acceptance Criteria (%)
Content of bacitracin A	NLT 40.0
Content of active bacitracin	NLT 70.0
Limit of early eluting peptides	NMT 20.0
Limit of bacitracin F	NMT 6.0

- **PH (791)**  
**Sample solution:** 10,000 Bacitracin Units/mL in water  
**Acceptance criteria:** 5.5–7.5
- **LOSS ON DRYING (731)**  
**Sample:** 100 mg  
**Analysis:** Dry the *Sample* in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.  
**Acceptance criteria:** NMT 5.0%
- **STERILITY TESTS (71):** Where the label states that the Bacitracin is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that the Bacitracin is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.01 USP Endotoxin Units/Bacitracin Unit.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 8°.
- **LABELING:** Where it is packaged for prescription compounding, label it to indicate that it is not sterile and that the potency cannot be assured for longer than 60 days after opening, and to state the number of Bacitracin Units/mg. Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.

**USP REFERENCE STANDARDS (11)**

- USP Bacitracin Zinc RS
- USP Endotoxin RS

**Bacitracin for Injection****DEFINITION**

Bacitracin for Injection has a potency of NLT 50 Bacitracin Units/mg. It contains NLT 90.0% and NMT 115.0% of the labeled amount of bacitracin.

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP):** Meets the requirements

**ASSAY****PROCEDURE**

(See *Antibiotics—Microbial Assays* (81).)

**Sample solution 1:** Nominally 100 Bacitracin Units/mL, prepared as follows. Constitute one container of Bacitracin for Injection as directed in the labeling. Using a suitable hypodermic needle and syringe, withdraw the contents of the container, and dilute with *Buffer B.1* (see the chapter) to a suitable volume.

**Sample solution 2** (where the label states the number of Bacitracin Units in a given volume of constituted solution): Nominally 100 Bacitracin Units/mL, prepared as follows. Constitute one container of Bacitracin for Injection as directed in the labeling. Dilute a suitable aliquot of the constituted solution with *Buffer B.1* (see the chapter) to a suitable final volume.

**Analysis**

**Samples:** *Sample solution 1* or *Sample solution 2*

Proceed as directed in the chapter. Add sufficient 0.01 N hydrochloric acid to the *Sample solution* so that the amount of hydrochloric acid in the *Test Dilution* is the same as in the median level of the standard. Dilute with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.

**Acceptance criteria:** 90.0%–115.0%

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

**IMPURITIES****RESIDUE ON IGNITION (281)**

**Analysis:** Moisten the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Acceptance criteria:** NMT 3.0%

**Delete the following:**

- **HEAVY METALS, Method II (231):** NMT 30 ppm (Official 1-Jan-2018)

**SPECIFIC TESTS**

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.
- **PH (791)**  
**Sample solution:** A solution containing 10,000 Bacitracin Units/mL  
**Acceptance criteria:** 5.5–7.5
- **LOSS ON DRYING (731)**  
**Analysis:** Dry 100 mg in a capillary-stoppered bottle under vacuum at a pressure of NMT 5 mm of mercury at 60° for 3 h.



- Acceptance criteria: NMT 5.0%
- STERILITY TESTS (71):** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.01 USP Endotoxin Unit/Bacitracin Unit.
- OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products (1)*

#### ADDITIONAL REQUIREMENTS

##### Change to read:

- PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements (659)*, *Injection Packaging, Packaging for constitution* (CN 1-May-2017), and store in a cool place.
- USP REFERENCE STANDARDS (11)**  
USP Bacitracin Zinc RS  
USP Endotoxin RS

### Bacitracin Ointment

#### DEFINITION

Bacitracin Ointment is Bacitracin in an anhydrous ointment base. It contains NLT 90.0% and NMT 140.0% of the labeled amount of bacitracin. It may contain a suitable anesthetic.

#### IDENTIFICATION

- A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP):** Meets the requirements

#### ASSAY

##### PROCEDURE

(See *Antibiotics—Microbial Assays (81)*.)

**Sample solution:** Use a portion of Ointment shaken with about 50 mL of ether in a separator and extracted with four 20-mL portions of *Buffer B.1* (see the chapter). Combine the buffer extracts, and dilute with *Buffer B.1* to a suitable volume.

**Analysis:** Proceed as directed in the chapter. Add sufficient 0.01 N hydrochloric acid to a suitable aliquot of the *Sample solution* so that the amount of hydrochloric acid in the *Test Dilution* is the same as in the median level of the standard. Dilute with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–140.0%

#### SPECIFIC TESTS

- WATER DETERMINATION, Method I (921)**  
**Analysis:** Use 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.  
Acceptance criteria: NMT 0.5%
- MINIMUM FILL (755):** Meets the requirements

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers containing NMT 60 g, unless labeled solely for hospital use, preferably at controlled room temperature.

- USP REFERENCE STANDARDS (11)**  
USP Bacitracin Zinc RS

### Bacitracin Ophthalmic Ointment

#### DEFINITION

Bacitracin Ophthalmic Ointment is a sterile preparation of Bacitracin in an anhydrous ointment base. It contains NLT 90.0% and NMT 140.0% of the labeled amount of bacitracin.

#### IDENTIFICATION

- A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP):** Meets the requirements

#### ASSAY

##### PROCEDURE

(See *Antibiotics—Microbial Assays (81)*.)

**Sample solution:** Use a portion of Ophthalmic Ointment shaken with about 50 mL of ether in a separator and extracted with four 20-mL portions of *Buffer B.1* (see the chapter). Combine the buffer extracts, and dilute with *Buffer B.1* to a suitable volume.

**Analysis:** Proceed as directed in the chapter. Add sufficient 0.01 N hydrochloric acid to a suitable aliquot of the *Sample solution* so that the amount of hydrochloric acid in the *Test Dilution* is the same as in the median level of the standard. Dilute with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–140.0%

#### SPECIFIC TESTS

- STERILITY TESTS (71):** Meets the requirements
- OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests (771)*.

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**  
USP Bacitracin Zinc RS

### Soluble Bacitracin Methylenedisalicylate

#### DEFINITION

Soluble Bacitracin Methylenedisalicylate is a mixture of Bacitracin Methylenedisalicylate and Sodium Bicarbonate. It has a potency of NLT 8 Bacitracin Units/mg, calculated on the dried basis.

#### ASSAY

##### Change to read:

- ANTIBIOTICS—MICROBIAL ASSAYS (81)**

**Diluent:** 20 g/L of sodium bicarbonate

**Sample stock solution:** Transfer a suitable amount of Soluble Bacitracin Methylenedisalicylate to a high-speed glass blender jar, add 99.0 mL of *Diluent* and 1.0 mL of polysorbate 80, and blend for 3 min.

**Test dilution:** To a suitable aliquot of the *Sample stock solution*, add a suitable volume of 0.01 N hydrochloric acid, and dilute with *Buffer B.1* (CN 1-May-2017) to obtain a concentration of bacitracin assumed to be equal to



the median dose level of the Standard. [NOTE—The amount of hydrochloric acid in the *Test dilution* should be the same as that in the median dose level of the Standard.]

**Analysis:** Proceed as directed for Bacitracin in *Antibiotics—Microbial Assays* (81).

**Acceptance criteria:** NLT 8 Bacitracin Units/mg on the dried basis

#### SPECIFIC TESTS

- **PH** (791): 8.0–9.5, in a 25 mg/mL solution
- **LOSS ON DRYING** (731): Dry 100 mg in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h; it loses NMT 8.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** (11)  
USP Bacitracin Zinc RS

### Bacitracin Methylenedisalicylate Soluble Powder

#### DEFINITION

Bacitracin Methylenedisalicylate Soluble Powder contains NLT 90.0% and NMT 120.0% of the labeled amount of bacitracin.

#### ASSAY

##### Change to read:

- **ANTIBIOTICS—MICROBIAL ASSAYS** (81)  
**Diluent:** 20 g/L of sodium bicarbonate  
**Sample stock solution:** Transfer a suitable amount of Bacitracin Methylenedisalicylate Soluble Powder to a high-speed glass blender jar, add 99.0 mL of *Diluent* and 1.0 mL of polysorbate 80, and blend for 3 min.  
**Test dilution:** To a suitable aliquot of the *Sample stock solution*, add a suitable volume of 0.01 N hydrochloric acid and dilute with *Buffer B.1* (CN 1-May-2017) to obtain a concentration of bacitracin assumed to be equal to the median dose level of the Standard. [NOTE—The amount of hydrochloric acid in the *Test dilution* should be the same as that in the median dose level of the Standard.]  
**Analysis:** Proceed as directed for Bacitracin in *Antibiotics—Microbial Assays* (81).  
**Acceptance criteria:** 90.0%–120.0%

#### SPECIFIC TESTS

- **PH** (791): 8.0–9.5 in a 50 mg/mL solution
- **LOSS ON DRYING** (731): Dry 100 mg in a capillary-stoppered bottle in a vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h; it loses NMT 8.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is for veterinary use only. Label it to state the content of bacitracin in terms of grams per pound, each gram of bacitracin being equivalent to 42,000 Bacitracin Units.

- **USP REFERENCE STANDARDS** (11)  
USP Bacitracin Zinc RS

### Bacitracin and Polymyxin B Sulfate Topical Aerosol

#### DEFINITION

Bacitracin and Polymyxin B Sulfate Topical Aerosol is a suspension of Bacitracin and Polymyxin B Sulfate in a suitable vehicle, packaged in a pressurized container with a suitable inert propellant. It contains NLT 90.0% and NMT 130.0% of the labeled amounts of bacitracin and polymyxin B. It may contain a suitable local anesthetic. Prepare the specimen for the following tests and assays as follows. Maintain the container in the inverted position throughout this procedure. Store the container in a freezer at –70° for 16–24 h. Remove the container from the freezer, promptly puncture the container, and allow the propellant to volatilize. Open the container, and mix the contents.

#### IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201BNP)  
**Sample:** Prepare as directed above.  
**Analysis:** Test as directed in the section *For Creams, Lotions, and Ointments* in the chapter.  
**Acceptance criteria:** Meets the requirements

#### ASSAY

- **BACITRACIN**  
(See *Antibiotics—Microbial Assays* (81).)  
**Sample solution:** Use a portion of the contents of one container, containing nominally 500 USP Bacitracin Units, prepared as directed above. Transfer to a suitable separator containing 50 mL of ether, and extract with three 25-mL portions of *Buffer B.1* (see the chapter). Combine the buffer extracts in a 100-mL volumetric flask, dilute with *Buffer B.1* to volume, and mix.  
**Analysis:** Proceed as directed in the chapter. Add sufficient 0.01 N hydrochloric acid to this solution so that the amount of hydrochloric acid in the *Test Dilution* is the same as in the median level of the standard. Dilute with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.  
**Acceptance criteria:** 90.0%–130.0%
- **POLYMYXIN B**  
(See *Antibiotics—Microbial Assays* (81).)  
**Sample solution:** Use a portion of the contents of one container, containing nominally 5000 USP Polymyxin B Units, prepared as directed above. Transfer to a suitable separator containing 50 mL of ether, and extract with three 25-mL portions of *Buffer B.6* (see the chapter). Combine the buffer extracts in a 100-mL volumetric flask, dilute with *Buffer B.6* to volume, and mix.  
**Analysis:** Proceed as directed in the chapter. Dilute a suitable aliquot of the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a polymyxin B concentration that is nominally equivalent to the median level of the standard.  
**Acceptance criteria:** 90.0%–130.0%

#### SPECIFIC TESTS

- **WATER DETERMINATION, Method I** (921)  
**Analysis:** Use a portion of the contents of one container, prepared as directed above, and 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.



Acceptance criteria: NMT 0.5%

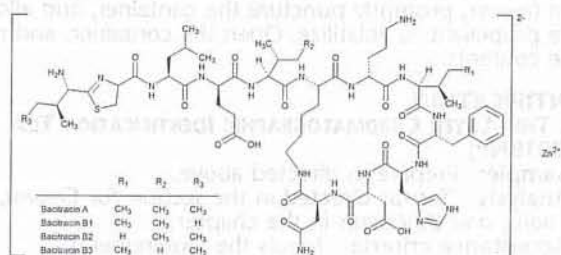
#### Change to read:

- **OTHER REQUIREMENTS:** It meets the requirements for **Topical Aerosols (603)** (CN 1-May-2017), in the sections **Pressure Test**, **Minimum Fill**, and **Leakage Test**.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in pressurized containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS (11)**  
USP Bacitracin Zinc RS  
USP Polymyxin B Sulfate RS

### Bacitracin Zinc



Bacitracins, zinc complex;  
Bacitracin zinc complex [1405-89-6].

#### DEFINITION

Bacitracin Zinc is the zinc complex of bacitracin, which consists of a mixture of antimicrobial polypeptides, the main components being bacitracins A, B1, B2, and B3. It has a potency of NLT 65 Bacitracin Units/mg, calculated on the dried basis. It contains NLT 4.0% and NMT 6.0% of zinc (Zn), calculated on the dried basis.

#### IDENTIFICATION

- **A.** Meets the requirements of the test for *Composition of Bacitracin*
- **B.** Meets the requirements of the test for *Zinc Content*

#### ASSAY

##### PROCEDURE

(See *Antibiotics—Microbial Assays (81)*.)

**Analysis:** Proceed as directed in the chapter.

**Acceptance criteria:** NLT 65 Bacitracin Units/mg on the dried basis

#### IMPURITIES

##### Delete the following:

- **RESIDUE ON IGNITION (281):** NMT 0.5% (ERR 1-Jun-2016)

#### SPECIFIC TESTS

##### COMPOSITION OF BACITRACIN

**Diluent:** 40 g/L of edetate disodium in water adjusted with 8 N sodium hydroxide to a pH of 7.0

**Solution A:** 34.8 g/L of dibasic potassium phosphate in water

**Solution B:** 27.2 g/L of monobasic potassium phosphate in water

**Solution C:** *Solution B* and *Solution A* (9:2). The pH of the mixture is about 6.

**Solution D:** 0.1 mM edetate disodium in a mixture of *Solution C* and water (1:3)

**Solution E:** Methanol and acetonitrile (27:2)

**Mobile phase:** *Solution E* and *Solution D* (63:37)

**System suitability solution:** 2 mg/mL of USP Bacitracin Zinc RS in *Diluent*

**Reporting threshold solution:** 0.01 mg/mL of USP Bacitracin Zinc RS from *System suitability solution* in water

**Peak identification solution:** 2 mg/mL of USP Bacitracin Zinc RS in *Diluent*. Heat in a boiling water bath for 30 min, and cool to room temperature.

**Sample solution:** 2 mg/mL of Bacitracin Zinc in *Diluent*

#### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm and 300 nm. Quantitative analysis is performed at 254 nm; 300 nm is only used to identify the location of bacitracin F.

**Column:** 4.6-mm × 25-cm; end-capped 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 100 μL

**Run time:** NLT 3 times the retention time of bacitracin A

#### System suitability

**Samples:** *System suitability solution* and *Peak identification solution*

Analyze the *Peak identification solution* at 300 nm. Identify bacitracin F, a known impurity, using the relative retention time provided in *Table 1*. Analyze the *System suitability solution* at 254 nm. Identify the peaks of the most active components of bacitracin (bacitracins A, B1, B2, and B3), early eluting peptides (those eluting before the peak due to bacitracin B1), and the impurity (bacitracin F) using the relative retention time values in *Table 1*.

Table 1

Name	Nature of Component	Relative Retention Time
Bacitracin C1	Early eluting peptides	0.5
Bacitracin C2		0.6
Bacitracin C3		0.6
Bacitracin B1	Active bacitracin	0.7
Bacitracin B2		0.7
Bacitracin B3		0.8
Bacitracin A		1.0
Bacitracin F	Impurity	2.4

#### Suitability requirements

**Peak-to-valley ratio:** NLT 1.2

The *Peak-to-valley ratio* is calculated as follows:

$$\text{Result} = H_P/H_V$$

$H_P$  = height above the baseline of the peak due to bacitracin B1

$H_V$  = height above the baseline of the lowest point of the curve separating the bacitracin B1 peak from the bacitracin B2 peak

#### Analysis

**Samples:** *Diluent*, *Reporting threshold solution*, and *Sample solution*

Quantitative analysis is performed at 254 nm.

#### Content of bacitracin A

Calculate the percentage of bacitracin A in the portion of Bacitracin Zinc taken:

$$\text{Result} = (r_A/r_T) \times 100$$

$r_A$  = peak area of bacitracin A from the *Sample solution*



$r_T$  = sum of all peak areas above the reporting threshold from the *Sample solution*

#### Content of active bacitracin

Calculate the percentage of active bacitracin (bacitracin A, B1, B2, and B3) in the portion of Bacitracin Zinc taken:

$$\text{Result} = [(r_A + r_{B1} + r_{B2} + r_{B3})/r_T] \times 100$$

$r_A$  = peak area of bacitracin A from the *Sample solution*

$r_{B1}$  = peak area of bacitracin B1 from the *Sample solution*

$r_{B2}$  = peak area of bacitracin B2 from the *Sample solution*

$r_{B3}$  = peak area of bacitracin B3 from the *Sample solution*

$r_T$  = sum of all peak areas above the reporting threshold from the *Sample solution*

#### Limit of early eluting peptides

Calculate the percentage of early eluting peptides (peaks eluting before bacitracin B1) in the portion of Bacitracin Zinc taken:

$$\text{Result} = (r_P/r_T) \times 100$$

$r_P$  = sum of peak areas for all peaks before bacitracin B1 from the *Sample solution*

$r_T$  = sum of all peak areas above the reporting threshold from the *Sample solution*

#### Limit of bacitracin F

Calculate the percentage of bacitracin F in the portion of Bacitracin Zinc taken:

$$\text{Result} = (r_F/r_A) \times 100$$

$r_F$  = peak area of bacitracin F from the *Sample solution*

$r_A$  = peak area of bacitracin A from the *Sample solution*

**Acceptance criteria:** See Table 2. Disregard any peaks from the *Sample solution* that are observed in the *Diluent* chromatogram. Disregard any peaks from the *Sample solution* having a peak area less than bacitracin A in the *Reporting threshold solution*.

Table 2

	Acceptance Criteria (%)
Content of bacitracin A	NLT 40.0
Content of active bacitracin	NLT 70.0
Limit of early eluting peptides	NMT 20.0
Limit of bacitracin F	NMT 6.0

#### • ZINC CONTENT

[NOTE—The *Standard solutions* and the *Sample solution* may be quantitatively diluted with 1 mM hydrochloric acid, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

**Standard stock solution:** 10 mg/mL of zinc from zinc oxide in 1 N hydrochloric acid. Prepare as follows.

Transfer a suitable amount of zinc oxide to a suitable volumetric flask, add 1 N hydrochloric acid using 32% of the final volume, warm to dissolve, cool and dilute with water to volume.

**Standard solutions:** 0.5, 1.5, and 2.5 µg/mL of zinc from *Standard stock solution* in 0.001 N hydrochloric acid

**Sample stock solution:** 2 mg/mL of Bacitracin Zinc in 0.01 N hydrochloric acid

**Sample solution:** 0.02 mg/mL of Bacitracin Zinc from *Sample stock solution* in 0.001 N hydrochloric acid

#### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 213.8 nm

**Lamp:** Zinc hollow-cathode

**Flame:** Air-acetylene

**Blank:** 0.001 N hydrochloric acid

#### Analysis

**Samples:** *Standard solutions*, *Sample solution*, and *Blank*  
Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of zinc, and draw the straight line best fitting the three plotted points. From the graph, determine the concentration, in µg/mL, of zinc in the *Sample solution*.

Calculate the percentage of zinc in the portion of Bacitracin Zinc taken:

$$\text{Result} = C \times D \times (V/W) \times F \times 100$$

$C$  = concentration of zinc in the *Sample solution* obtained from the curve (µg/mL)

$D$  = dilution factor for the *Sample solution*, 100 mL/mL

$V$  = volume of *Sample stock solution* (mL)

$W$  = weight of Bacitracin Zinc used to prepare the *Sample stock solution* (mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** 4.0%–6.0% on the dried basis

#### • PH (791)

**Sample solution:** A saturated solution in water containing about 100 mg/mL

**Acceptance criteria:** 6.0–7.5

#### • LOSS ON DRYING (731)

**Sample:** 100 mg

**Analysis:** Dry the *Sample* in a capillary-stoppered bottle under vacuum at 60° for 3 h.

**Acceptance criteria:** NMT 5.0%

#### • STERILITY TESTS (71):

Where the label states that it is sterile, it meets the requirements of the chapter. If the membrane filtration test is used, add 20 g of edetate disodium to each L of *Fluid A*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 25°.
- **LABELING:** Label it to indicate that it is to be used in the manufacture of nonparenteral drugs only. Where it is packaged for prescription compounding, label it to indicate that it is not sterile and that the potency cannot be assured for longer than 60 days after opening, and to state the number of Bacitracin Units/mg. Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.
- **USP REFERENCE STANDARDS (11)**  
USP Bacitracin Zinc RS

## Bacitracin Zinc Ointment

#### DEFINITION

Bacitracin Zinc Ointment is Bacitracin Zinc in an anhydrous ointment base. It contains NLT 90.0% and NMT 140.0% of the labeled amount of bacitracin.

#### IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP):** Meets the requirements



**ASSAY**• **PROCEDURE**(See *Antibiotics—Microbial Assays* (81).)

**Standard solution:** Proceed as directed in the chapter. To each *Test Dilution* of the standard add sufficient hydrochloric acid to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of Ointment.

**Sample solution:** Use a portion of Ointment shaken with about 50 mL of ether in a separator and extracted with four 20-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.

**Analysis:** Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard. Add sufficient hydrochloric acid to each *Test Dilution* of the standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of the sample.

**Acceptance criteria:** 90.0%–140.0%

**SPECIFIC TESTS**• **WATER DETERMINATION, Method I (921)**

**Analysis:** Use 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.

**Acceptance criteria:** NMT 0.5%

• **MINIMUM FILL (755):** Meets the requirements**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers containing NMT 60 g, unless labeled solely for hospital use, preferably at controlled room temperature.• **USP REFERENCE STANDARDS (11)**

USP Bacitracin Zinc RS

**Bacitracin Zinc Soluble Powder**

» Bacitracin Zinc Soluble Powder is a mixture of Bacitracin Zinc and zinc proteinates. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of bacitracin.

**Packaging and storage—**Preserve in tight containers.

**Labeling—**Label it to indicate that it is for veterinary use only. Label it to state the content of bacitracin in terms of grams per pound, each gram of bacitracin being equivalent to 42,000 Bacitracin Units.

**USP Reference standards (11)—**

USP Bacitracin Zinc RS

**Loss on drying (731)—**Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 5.0% of its weight.

**Zinc content—**Using Powder, proceed as directed for *Zinc content* under *Bacitracin Zinc*. Calculate the zinc content, in g, in relation to each 42,000 Bacitracin Units in the specimen taken by the formula:

$$\frac{280,000C}{WA}$$

in which A is the bacitracin content of the specimen, in Bacitracin Units per g, and the other terms are as defined therein: it contains not more than 2.0 g for each 42,000 Bacitracin Units.

**Change to read:**

**Assay—**Dissolve an accurately weighed quantity of Powder quantitatively in 0.01 N hydrochloric acid to obtain a stock solution containing about 100 Bacitracin Units per mL. Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this stock solution diluted quantitatively and stepwise with *Buffer B.1* (CN 1: May 2017) to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard. In preparing each test dilution of the Standard, add additional hydrochloric acid to each to obtain the same concentration of hydrochloric acid as in the *Test Dilution*.

**Bacitracin Zinc and Polymyxin B Sulfate Ointment****DEFINITION**

Bacitracin Zinc and Polymyxin B Sulfate Ointment contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amounts of bacitracin and polymyxin B. It may contain a suitable local anesthetic.

**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP):** Meets the requirements**ASSAY**• **BACITRACIN**(See *Antibiotics—Microbial Assays* (81).)

**Standard solution:** Proceed as directed in the chapter. To each *Test Dilution* of the standard add sufficient hydrochloric acid to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of Ointment.

**Sample solution:** Shake a portion of Ointment with about 50 mL of ether in a separator, and extract with four 20-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.

**Analysis:** Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard. Add sufficient hydrochloric acid to each *Test Dilution* of the standard to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of the sample.

**Acceptance criteria:** 90.0%–130.0%

• **POLYMYXIN B**(See *Antibiotics—Microbial Assays* (81).)

**Sample solution:** Shake a portion of Ointment with about 50 mL of ether in a separator, and extract with four 20-mL portions of *Buffer B.6* (see the chapter). Combine the buffer extracts, and dilute with *Buffer B.6* to a suitable volume.

**Analysis:** Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a polymyxin B concentration that is nominally equivalent to the median level of the standard.

**Acceptance criteria:** 90.0%–130.0%

**SPECIFIC TESTS**• **WATER DETERMINATION, Method I (921)**

**Analysis:** Use 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.

**Acceptance criteria:** NMT 0.5%

• **MINIMUM FILL (755):** Meets the requirements**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.



- **USP REFERENCE STANDARDS (11)**  
USP Bacitracin Zinc RS  
USP Polymyxin B Sulfate RS

## Bacitracin Zinc and Polymyxin B Sulfate Ophthalmic Ointment

### DEFINITION

Bacitracin Zinc and Polymyxin B Sulfate Ophthalmic Ointment contains the equivalent of NLT 90.0% and NMT 130.0% of the labeled amounts of bacitracin and polymyxin B.

### IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201BNP):** Meets the requirements

### ASSAY

#### • BACITRACIN

(See *Antibiotics—Microbial Assays* (81).)

**Standard solution:** Proceed as directed in the chapter. To each *Test Dilution* of the standard add sufficient hydrochloric acid to obtain the same concentration of hydrochloric acid as in the *Test Dilution* of Ophthalmic Ointment.

**Sample solution:** Use a portion of Ophthalmic Ointment shaken with about 50 mL of ether in a separator and extracted with four 20-mL portions of 0.01 N hydrochloric acid. Combine the acid extracts, and dilute with 0.01 N hydrochloric acid to a suitable volume.

**Analysis:** Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.1* to obtain a *Test Dilution* having a bacitracin concentration that is nominally equivalent to the median level of the standard.

**Acceptance criteria:** 90.0%–130.0%

#### • POLYMYXIN B

(See *Antibiotics—Microbial Assays* (81).)

**Sample solution:** Shake a portion of Ophthalmic Ointment with about 50 mL of ether in a separator, and extract with four 20-mL portions of *Buffer B.6* (see the chapter). Combine the buffer extracts, and dilute with *Buffer B.6* to a suitable volume.

**Analysis:** Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a polymyxin B concentration that is nominally equivalent to the median level of the standard.

**Acceptance criteria:** 90.0%–130.0%

### SPECIFIC TESTS

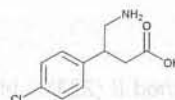
- **STERILITY TESTS (71):** Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at controlled room temperature.

- **USP REFERENCE STANDARDS (11)**  
USP Bacitracin Zinc RS  
USP Polymyxin B Sulfate RS

## Baclofen



$C_{10}H_{12}ClNO_2$  213.66  
Butanoic acid, 4-amino-3-(4-chlorophenyl)-;  
 $\beta$ -(Aminomethyl)-*p*-chlorohydrocinnamic acid [1134-47-0].

### DEFINITION

Baclofen contains NLT 98.0% and NMT 102.0% of baclofen ( $C_{10}H_{12}ClNO_2$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Solution A:** Dissolve 1.38 g of potassium dihydrogen phosphate and 1.74 g of sodium-1-pentanesulfonate in 1 L of water. Adjust with dilute phosphoric acid to a pH of 3.0.

**Solution B:** Acetonitrile and methanol (1:1)

**Diluent:** *Solution A* and *Solution B* (65:35)

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	65	35
5	65	35
15	45	55
25	45	55
27	65	35
30	65	35

**Standard solution:** 0.2 mg/mL of USP Baclofen RS in *Diluent*

**Sample solution:** 0.2 mg/mL of Baclofen in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 225 nm

**Column:** 4.6-mm  $\times$  25.0-cm; 5- $\mu$ m packing L1

**Column temperature:** 35°

**Flow rate:** 0.8 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of baclofen ( $C_{10}H_{12}ClNO_2$ ) in the portion of the Baclofen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*



$C_S$  = concentration of USP Baclofen RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Baclofen in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.3%

#### Delete the following:

- **HEAVY METALS**, Method II (231): NMT 10 ppm (Official 1-Jan-2018)

#### ORGANIC IMPURITIES

*Solution A*, *Solution B*, *Diluent*, *Mobile phase*, and *Chromatographic system*: Proceed as directed in the *Assay*.

**Standard solution:** 0.0015 mg/mL of USP Baclofen RS and 0.003 mg/mL of USP Baclofen Related Compound A RS in *Diluent*

**Sample solution:** 0.3 mg/mL of Baclofen in *Diluent*

**System suitability**

**Sample:** *Standard solution*

[NOTE—See Table 2 for relative retention times.]

**Suitability requirements**

**Tailing factor:** NMT 1.5 for baclofen

**Relative standard deviation:** NMT 5.0% for both baclofen and baclofen related compound A

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of baclofen related compound A in the portion of the Baclofen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of baclofen related compound A from the *Sample solution*

$r_S$  = peak response of baclofen related compound A from the *Standard solution*

$C_S$  = concentration of USP Baclofen Related Compound A RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Baclofen in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of the Baclofen taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity from the *Sample solution*

$r_S$  = peak response of baclofen from the *Standard solution*

$C_S$  = concentration of USP Baclofen RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Baclofen in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Baclofen	1.0	—
Baclofen related compound A	2.3	1.0
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

#### SPECIFIC TESTS

- **WATER DETERMINATION**, Method I (921): NMT 3.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.

- **USP REFERENCE STANDARDS** (11)

USP Baclofen RS

USP Baclofen Related Compound A RS

4-(4-Chlorophenyl)-2-pyrrolidinone.

C<sub>10</sub>H<sub>10</sub>ClNO 195.65

## Baclofen Compounded Oral Suspension

#### DEFINITION

Baclofen Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of baclofen (C<sub>10</sub>H<sub>12</sub>ClNO<sub>2</sub>).

Prepare Baclofen Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Baclofen	500 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

If using *Baclofen Tablets*, place the Tablets in a suitable mortar and comminute to a fine powder, or add *Baclofen* powder. Add 5 mL of the *Vehicle* to wet the powder, and triturate the powder to form a fine paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer, stepwise and quantitatively, the contents of the mortar to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

#### ASSAY

##### PROCEDURE

**Mobile phase:** Acetonitrile and 0.05 M monobasic sodium phosphate (20:80). Adjust with phosphoric acid to a pH of 3.5.

**Standard solution:** 5 µg/mL of USP Baclofen RS in water

**Sample solution:** Shake thoroughly by hand each bottle of Oral Suspension. Pipet 0.5 mL of Oral Suspension from each bottle to a 500-mL volumetric flask, dilute with water to volume to obtain a concentration of 5 µg/mL, and pass through a 0.22-µm polyvinylidene fluoride (PVDF) filter.

##### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 15 µL

##### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time of baclofen is about 5.5 min.]

##### Suitability requirements

**Relative standard deviation:** NMT 2.0% for replicate injections

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of baclofen (C<sub>10</sub>H<sub>12</sub>ClNO<sub>2</sub>) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Baclofen RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of baclofen in the *Sample solution* ( $\mu\text{g/mL}$ )  
 Acceptance criteria: 90.0%–110.0%

**SPECIFIC TESTS**

- **PH (791):** 4.2–5.2

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator.
- **BEYOND-USE DATE:** NMT 35 days after the day on which it was compounded when stored in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS (11)**  
USP Baclofen RS

**Baclofen Tablets****DEFINITION**

Baclofen Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of baclofen ( $\text{C}_{10}\text{H}_{12}\text{ClNO}_2$ ).

**IDENTIFICATION**

- **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Diluent:** Methanol, water, and glacial acetic acid (30:66:4)

**Mobile phase:** Methanol, 0.3 N acetic acid, and 0.36 N sodium 1-pentanesulfonate (44:55:2)

**Standard solution:** 4 mg/mL of USP Baclofen RS in *Diluent*

**Sample solution:** Weigh and finely powder NLT 20 Tablets. Transfer an amount equivalent to 40 mg to a 50-mL flask. Add 10.0 mL of *Diluent* to the flask. Sonicate to disperse, and shake by mechanical means for 30 min. Centrifuge a portion of this solution for 5 min, and filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu\text{m}$  packing L1

**Flow rate:** 0.6 mL/min

**Injection volume:** 10  $\mu\text{L}$

**Run time:** NLT 3 times the retention time of baclofen

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

Relative standard deviation: NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of labeled amount of baclofen ( $\text{C}_{10}\text{H}_{12}\text{ClNO}_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Baclofen RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**

- **DISSOLUTION, Procedure for a Pooled Sample (711)**

**Medium:** 0.01 N hydrochloric acid; 500 mL for Tablets containing NMT 10 mg of baclofen; 1000 mL for Tablets containing more than 10 mg of baclofen

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Mobile phase:** Proceed as directed in the *Assay*.

**Standard solution:** USP Baclofen RS in *Medium*

**Sample solution:** Sample per *Dissolution* (711).

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu\text{m}$  packing L1

**Flow rate:** 0.6 mL/min

**Injection volume:** 190  $\mu\text{L}$

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

Relative standard deviation: NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of baclofen ( $\text{C}_{10}\text{H}_{12}\text{ClNO}_2$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Baclofen RS in the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*; 500 or 1000 mL  
 $L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of baclofen ( $\text{C}_{10}\text{H}_{12}\text{ClNO}_2$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES****• ORGANIC IMPURITIES**

**Diluent, Mobile phase, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard stock solution:** 1 mg/mL of USP Baclofen Related Compound A RS in methanol

**Standard solution:** 0.16 mg/mL of USP Baclofen Related Compound A RS in *Diluent* from the *Standard stock solution*

**Analysis**

[NOTE—The elution order is baclofen followed by baclofen related compound A.]

**Samples:** *Sample solution* and *Standard solution*  
Calculate the percentage of baclofen related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak area response from the *Sample solution*  
 $r_S$  = peak area response from the *Standard solution*  
 $C_S$  = concentration of USP Baclofen Related Compound A RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of baclofen in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 4.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.



• **USP REFERENCE STANDARDS** (11)

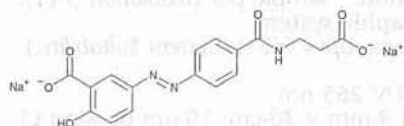
USP Baclofen RS

USP Baclofen Related Compound A RS

4-(4-Chlorophenyl)-2-pyrrolidinone.

C<sub>10</sub>H<sub>10</sub>ClNO 195.65

## Balsalazide Disodium



C<sub>17</sub>H<sub>13</sub>N<sub>3</sub>Na<sub>2</sub>O<sub>6</sub> · 2H<sub>2</sub>O 437.31

Benzoic acid, 5-[[4-[(2-carboxyethyl)amino]carbonyl]phenyl]azo]-2-hydroxy-, disodium salt, dihydrate, (E)-; (E)-5-[[p-[(2-Carboxyethyl)carbamoyl]phenyl]azo]salicylic acid, disodium salt, dihydrate [150399-21-6].

### DEFINITION

Balsalazide Disodium contains NLT 98.0% and NMT 102.0% of C<sub>17</sub>H<sub>13</sub>N<sub>3</sub>Na<sub>2</sub>O<sub>6</sub> · 2H<sub>2</sub>O, calculated on the as-is basis.

### IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B. ULTRAVIOLET ABSORPTION** (197U)

Sample solution: 10 µg/mL in water

• **C. IDENTIFICATION TESTS—GENERAL**, Sodium (191)

### ASSAY

#### PROCEDURE

Sample: 219 mg

Analysis: Add 80 mL of glacial acetic acid to the Sample, sonicate to dissolve, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 21.87 mg of C<sub>17</sub>H<sub>13</sub>N<sub>3</sub>Na<sub>2</sub>O<sub>6</sub> · 2H<sub>2</sub>O.

Acceptance criteria: 98.0%–102.0% on the as-is basis

### IMPURITIES

#### Inorganic Impurities

#### Delete the following:

• **HEAVY METALS**, Method II (231): NMT 20 ppm (Official 1-Jan-2018)

#### Organic Impurities

[NOTE—On the basis of the synthetic route, perform either Procedure 1 or Procedure 2. Procedure 2 is recommended when impurities 1, 2, and 3 listed in *Impurity Table 2* may be present.]

#### PROCEDURE 1

**Solution A:** Dissolve 2.7 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 10% potassium hydroxide solution to a pH of 6.00 ± 0.1.

**Diluent:** Use *Solution A*.

**Solution B:** Use acetonitrile.

**Standard solution:** 0.5 µg/mL of USP Balsalazide Disodium RS, 0.5 µg/mL of USP Balsalazide Related Compound A RS, 0.5 µg/mL of USP Balsalazide Related Compound B RS, and 0.5 µg/mL of USP Salicylic Acid RS in *Diluent*. If needed, a small amount of acetonitrile may be added to facilitate dissolution. [NOTE—USP Balsalazide Related Compound A RS is the disodium salt of (E)-5-[(p-carboxyphenyl)azo]-2-salicylic acid. Use the correction factor stated on the label of the USP Reference Standard to calculate the concentration, as appropriate.]

**Sample solution:** 1 mg/mL of Balsalazide Disodium in *Diluent*

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
40	75	25
47	75	25
55	50	50
60	50	50
60.1	90	10
70	90	10

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 238 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 45°

**Flow rate:** 1 mL/min

**Injection size:** 30 µL

### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 5 between balsalazide and balsalazide related compound B

**Relative standard deviation:** NMT 5% for each peak

**Tailing factor:** NMT 1.8 for the balsalazide peak

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Balsalazide Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for each individual impurity from the *Sample solution*

$r_S$  = peak response for the corresponding impurity from the *Standard solution*. [NOTE—For unspecified impurities,  $r_S$  is the peak response for the balsalazide peak from the *Standard solution*.]

$C_S$  = concentration of the corresponding impurity in the *Standard solution* (mg/mL). [NOTE—For unspecified impurities,  $C_S$  is the concentration of balsalazide disodium in the *Standard solution*.]

$C_U$  = concentration of Balsalazide Disodium in the *Sample solution* (mg/mL)

### Acceptance criteria

**Individual impurities:** See *Impurity Table 1*.

**Reporting level for impurities:** 0.03%

**Total impurities:** NMT 1.0%

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Salicylic acid	0.37	0.05
Balsalazide related compound A <sup>a</sup>	0.70	0.05
Balsalazide	1.00	—

<sup>a</sup> (E)-5-[(p-Carboxyphenyl)azo]-2-salicylic acid.

<sup>b</sup> (E)-5-[(m-[(2-Carboxyethyl)carbamoyl]phenyl)azo]-2-salicylic acid.



Impurity Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Balsalazide related compound B <sup>a</sup>	1.2	0.05
Any other individual unspecified impurity	—	0.05

<sup>a</sup> (E)-5-[(p-Carboxyphenyl)azo]-2-salicylic acid.<sup>b</sup> (E)-5-[(m-[(2-Carboxyethyl)carbamoyl]phenyl)azo]-2-salicylic acid.**PROCEDURE 2**

**Solution A:** Prepare 50 mM monobasic potassium phosphate buffer as follows: Dissolve 6.8 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 2 N potassium hydroxide solution to a pH of 6.8–7.0. [NOTE—To ensure proper identification of impurity 1, the pH must be maintained between 6.8 and 7.0.]

**Solution B:** Acetonitrile, methanol and *Solution A* (5:1:14)

**Solution C:** Acetonitrile, methanol and *Solution A* (9:1:10)

**Diluent:** Use *Solution B*.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	100	0	0
37	0	100	0
60	0	0	100
75	100	0	0
85	100	0	0

**Standard solution:** 0.075 mg/mL of USP Balsalazide Disodium RS in *Diluent*. [NOTE—Use sonication to dissolve.]

**Sensitivity solution:** 0.375 µg/mL in *Diluent*, from *Standard solution*

**System suitability solution:** 1.5 mg/mL of USP Balsalazide Disodium RS and 1.5 µg/mL of USP Balsalazide Related Compound A RS in *Diluent*

**Sample solution:** 1.5 mg/mL of Balsalazide Disodium in *Diluent*. [NOTE—Use sonication to dissolve.]

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Column:** 4.6-mm × 15-cm; 3-µm packing L1

**Column temperature:** 25°–27°. [NOTE—To ensure proper identification of impurity 1, the column temperature must be maintained between 25° and 27°.]

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Samples:** *Standard solution*, *Sensitivity solution*, and *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 8.5 between balsalazide related compound A and balsalazide, from the *System suitability solution*

**Tailing factor:** NMT 3.4 for the balsalazide peak, from the *System suitability solution*

**Signal-to-noise ratio:** NLT 10, from the *Sensitivity solution*

**Relative standard deviation:** NMT 2.0%, from the *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Balsalazide Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each individual impurity from the *Sample solution*

$r_S$  = peak response for balsalazide from the *Standard solution*

$C_S$  = concentration of USP Balsalazide Disodium RS in the *Standard solution*

$C_U$  = concentration of Balsalazide Disodium in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 2*)

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 2*.

**Reporting level for impurities:** 0.03%

**Total impurities:** NMT 0.50%

Impurity Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
N-(4-Aminobenzoyl)-β-alanine <sup>a</sup>	0.29	1.8	0.05
Salicylic acid	0.55	1.4	0.05
Balsalazide related compound A <sup>b</sup>	0.88	1.4	0.05
Impurity 1 <sup>c</sup>	0.91	1.9	0.05
Impurity 2 <sup>d</sup>	0.92	1.4	0.05
Impurity 3 <sup>e</sup>	0.94	0.83	0.05
Balsalazide	1.00	—	—
Impurity 4 <sup>f</sup>	1.35	2.1	0.05
Impurity 5 <sup>g</sup>	1.77	0.91	0.05
Any other individual unspecified impurity	—	—	0.05

<sup>a</sup> This impurity may be present as two peaks. Use the sum of the two peaks to determine compliance.

<sup>b</sup> (E)-5-[(p-Carboxyphenyl)azo]-2-salicylic acid.

<sup>c</sup> (E,E)-3,5-di-[4-(2-Carboxyethylcarbamoyl)phenylazo]-salicylic acid.

<sup>d</sup> (E)-3-[4-(2-Carboxyethylcarbamoyl)phenylazo]-salicylic acid.

<sup>e</sup> (E,E)-5-[(2-[4-(2-Carboxyethylcarbamoyl)phenylazo]-4-[2-carboxyethylcarbamoyl]]phenylazo)-salicylic acid.

<sup>f</sup> (E)-2-[4-(2-Carboxyethylcarbamoyl)phenoxy]-5-[[4-(2-carboxyethylcarbamoyl)]phenylazo]-benzoic acid

<sup>g</sup> (E)-2-Hydroxy-5-[[4-[(3-isopropoxy-3-oxopropyl)amino]carbonyl]phenyl]azo]benzoic acid

**SPECIFIC TESTS**

• **WATER DETERMINATION, Method 1a** <921>: 7.8%–9.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **LABELING:** If a test for *Organic Impurities* other than *Test 1* is used, the labeling states the test with which the article complies.

• **USP REFERENCE STANDARDS** (11)

USP Balsalazide Disodium RS

USP Balsalazide Related Compound A RS

(E)-5-[(p-Carboxyphenyl)azo]-2-salicylic acid, disodium salt.

C<sub>14</sub>H<sub>8</sub>N<sub>2</sub>O<sub>5</sub>Na<sub>2</sub> 330.12



USP Balsalazide Related Compound B RS  
(*E*)-5-[(*m*-(2-Carboxyethyl)carbamoyl)phenyl]azo)-2-salicylic acid.  
 $C_{17}H_{13}N_3O_6$  357.17  
USP Salicylic Acid RS

## Balsalazide Disodium Capsules

### DEFINITION

Balsalazide Disodium Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of balsalazide disodium ( $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. ULTRAVIOLET ABSORPTION**  
Using a 0.2-cm cell, record the UV spectrum of the *Sample solution*, obtained in the *Assay*, in the range of 200–400 nm: it exhibits maxima at about 261 nm and 357 nm.

### ASSAY

#### • PROCEDURE

**Buffer:** Add 5 mL of triethylamine to 1000 mL of water, and adjust with phosphoric acid to a pH of  $6.00 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (1:4)

**Diluent:** Water

**Standard solution:** 60 µg/mL of USP Balsalazide Disodium RS in *Diluent*. [NOTE—Use sonication as necessary.]

**Sample stock solution:** Transfer an equivalent to 150 mg of balsalazide disodium, from the Capsules contents, to a 100-mL volumetric flask, add 70 mL of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume.

**Sample solution:** 60 µg/mL of balsalazide disodium, from the *Sample stock solution*, in *Diluent*. Pass a portion of this solution through a suitable filter, discarding the first 3 mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 360 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 10,000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Sample:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of  $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$  in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of balsalazide disodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

### IMPURITIES

#### Organic Impurities

##### • PROCEDURE

**Buffer:** Dissolve 2.7 g of monobasic potassium phosphate in 1000 mL of water, and adjust with 10% potassium hydroxide solution to a pH of  $6.00 \pm 0.1$ .

**Diluent:** Water

**Solution A:** *Buffer*

**Solution B:** Acetonitrile

**Sample solution:** Transfer an amount of finely crushed powder equivalent to 100 mg of balsalazide disodium to a 100-mL volumetric flask, add 70 mL of *Diluent*, sonicate for 5 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a PVDF filter of 0.45-µm pore size.

**Standard solution:** 1.0 µg/mL of USP Balsalazide Disodium RS in *Diluent*

**System suitability solution:** 1.0 µg/mL of USP Balsalazide Disodium RS, 1.5 µg/mL of USP Balsalazide Related Compound A RS, 0.5 µg/mL of USP Balsalazide Related Compound B RS, and 0.5 µg/mL of USP Salicylic Acid RS in *Diluent*

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4	90	10
40	75	25
47	75	25
55	50	50
60	50	50
60.1	90	10
70	90	10

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 238 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 45°

**Flow rate:** 1 mL/min

**Injection size:** 30 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 5 between balsalazide and balsalazide related compound B, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Tailing factor:** NMT 1.5, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each individual impurity from the *Sample solution*

$r_S$  = peak response for the balsalazide peak from the *Standard solution*

$C_S$  = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of balsalazide disodium in the *Sample solution*, based on the label claim (mg/mL)

$F$  = relative response factor (see *Impurity Table 1*)



**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*.

**Reporting level for impurities:** 0.05%

**Total impurities:** NMT 1.0%. [NOTE—When reporting results for *Individual impurities* and *Total impurities*, disregard peaks corresponding to salicylic acid and balsalazide related compound B, as these impurities are controlled in the drug substance only.]

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Salicylic acid	0.37	—	—
Balsalazide related compound A <sup>a</sup>	0.70	1.3	0.15
Balsalazide	1.00	—	—
Balsalazide related compound B <sup>b</sup>	1.2	—	—
Any other individual unspecified impurity	—	1.0	0.10

<sup>a</sup> (E)-5-[(p-Carboxyphenyl)azo]-2-salicylic acid.

<sup>b</sup> (E)-5-[(m-[(2-Carboxyethyl)carbamoyl]phenyl)azo]-2-salicylic acid.

**PERFORMANCE TESTS****• DISSOLUTION (711)**

**Medium:** pH 6.8 phosphate buffer; 900 mL

**Apparatus 2:** 50 rpm, with stainless steel wire helix sinkers

**Time:** 30 min

**Detector:** UV 357 nm, with background correction at 590 nm

**Path length:** 0.02-cm flow cell

**Blank:** *Medium*

**Standard solution:** 0.83 mg/mL of USP Balsalazide Disodium RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 20-μm pore size.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of  $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$  dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (100/L)$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Balsalazide Disodium RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium* (mL), 900

$L$  = Capsule label claim (mg)

**Tolerances:** NLT 70% (Q) of the labeled amount of  $C_{17}H_{13}N_3Na_2O_6 \cdot 2H_2O$  is dissolved.

**• UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.**• USP REFERENCE STANDARDS (11)**

USP Balsalazide Disodium RS

USP Balsalazide Related Compound A RS

(E)-5-[(p-Carboxyphenyl)azo]-2-salicylic acid, disodium salt.

$C_{14}H_8N_2O_5Na_2$  330.12

USP Balsalazide Related Compound B RS

(E)-5-[(m-[(2-Carboxyethyl)carbamoyl]phenyl)azo]-2-salicylic acid.

$C_{17}H_{13}N_3O_6$  357.17

**USP Salicylic Acid RS****Adhesive Bandage**

» Adhesive Bandage consists of a compress of four layers of Type I Absorbent Gauze, or other suitable material, affixed to a film or fabric coated with a pressure-sensitive adhesive substance. It is sterile. The compress may contain a suitable antimicrobial agent and may contain one or more suitable colors. The adhesive surface is protected by a suitable removable covering.

**Packaging and storage—**Package Adhesive Bandage that does not exceed 15 cm (6 inches) in width individually in such manner that sterility is maintained until the individual package is opened. Package individual packages in a second protective container.

**Labeling—**The label of the second protective container bears a statement that the contents may not be sterile if the individual package has been damaged or previously opened, and it bears the names of any added antimicrobial agents. Each individual package is labeled to indicate the dimensions of the compress and the name of the manufacturer, packer, or distributor, and each protective container indicates also the address of the manufacturer, packer, or distributor.

**Sterility Tests (71):** meets the requirements.

**Gauze Bandage**

» Gauze Bandage is Type I Absorbent Gauze. Its length is not less than 98.0 percent of that declared on the label, and its average width is not more than 1.6 mm less than the declared width. It contains no dye or other additives.

**Packaging and storage—**Gauze Bandage that has been rendered sterile is so packaged that the sterility of the contents of the package is maintained until the package is opened for use.

**Labeling—**The width and length of the Bandage, the number of pieces contained, and the name of the manufacturer, packer, or distributor, are stated on the package. The designation "non-sterilized" or "not sterilized" appears prominently on the package unless the Gauze Bandage has been rendered sterile, in which case it may be labeled to indicate that it is sterile and that the contents may not be sterile if the package bears evidence of damage or if the package has been previously opened.

**NOTE—**Before determining the thread count, dimensions, and weight, hold the Bandage, unrolled, for not less than 4 hours in a standard atmosphere of  $65 \pm 2\%$  relative humidity at  $21 \pm 1.1^\circ \text{C}$  ( $70 \pm 2^\circ \text{F}$ ).

**Thread count—**Count the number of warp and filling threads of it in areas of 1.27 cm ( $1/2$  inch) square at 5 points evenly spread along the center line of the Bandage, no point being within 30.5 cm (12 inches) of either end of the Bandage, and calculate the average number of threads per 2.54 cm (1 inch) in each direction. A variation of not more than 3 threads per inch is allowed in either warp or filling, provided that the combined variations do not exceed 5 threads per square inch.



**Width**—Measure its width at each of the 5 points selected for the determination of the thread count: the average of 5 measurements is not more than 1.6 mm ( $1/16$  inch) less than the labeled width of the Bandage.

**Length**—Measure the length of the unrolled Gauze Bandage, smoothed without tension, along the center line of the Gauze Bandage: the length is not less than 98.0% of the labeled length of the Bandage.

**Weight**—Weigh the entire Bandage: the calculated weight in g per 0.894 square meter (1 linear yard *Type I gauze*), using the measurements obtained as described in the two paragraphs just preceding, is not less than 39.2 g.

**Absorbency**—Hold a rolled Gauze Bandage horizontal to and almost in contact with the surface of water at 25°, and allow it to drop lightly upon the water: complete submersion takes place in not more than 30 seconds.

**Sterility Tests** (71): Gauze Bandage that has been rendered sterile meets the requirements.

**Other requirements**—It meets the requirements of the tests for *Ignited residue*, *Acid or alkali*, and *Dextrin or starch*, in *water extract*, *Residue on ignition*, *Fatty matter*, and *Alcohol-soluble dyes* under *Absorbent Gauze*.

## Barium Hydroxide Lime

### DEFINITION

Barium Hydroxide Lime is a mixture of barium hydroxide octahydrate and Calcium Hydroxide. It may also contain Potassium Hydroxide and may contain an indicator that is inert toward anesthetic gases such as Ether, Cyclopropane, and Nitrous Oxide and that changes color when the Barium Hydroxide Lime no longer can absorb carbon dioxide.

[**CAUTION**—Because Barium Hydroxide Lime contains a soluble form of barium, it is toxic if swallowed.]

### IDENTIFICATION

- **A.** Analysis: Place a granule of it on a piece of moistened red litmus paper.  
Acceptance criteria: The paper turns blue immediately.
- **B. IDENTIFICATION TESTS—GENERAL** (191), *Barium*  
Sample solution: 100 mg/mL in 6 N acetic acid  
Acceptance criteria: Meets the requirements
- **C. IDENTIFICATION TESTS—GENERAL** (191), *Calcium*  
Sample solution: 100 mg/mL in 6 N acetic acid  
Acceptance criteria: Meets the requirements

### SPECIFIC TESTS

- **PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING** (786)  
Sample: 100 g  
Analysis: Screen the *Sample* for 5 min as directed in the chapter, using a mechanical shaker.  
Acceptance criteria: It passes completely through a No. 2 standard-mesh sieve, and NMT 2.0% passes through a No. 40 standard-mesh sieve. NMT 7.0% is retained on the coarse-mesh sieve, and NMT 15.0% passes through the fine-mesh sieve designated on the label.
- **LOSS ON DRYING** (731)  
Sample: 10 g  
Analysis: Weigh the *Sample* in a tared weighing bottle, and dry at 105° for 2 h.  
Acceptance criteria: 11.0%–16.0%
- **HARDNESS**  
Sample: 200 g  
Analysis: Screen the *Sample* on a mechanical sieve shaker (see (786)) with a frequency of oscillation of  $285 \pm 3$  cycles/min, for 3 min, to remove granules coarser than 4-mesh and finer than 8-mesh. Weigh 50 g

of the granules retained on the screen, and place them in a hardness pan that has a diameter of 200 mm and a concave brass bottom. The bottom of the pan is 7.9 mm thick at the circumference and 3.2 mm thick at the center and has an inside spherical radius of curvature of 109 cm. Add 15 steel balls of 7.9-mm diameter, and shake on a mechanical sieve shaker for 30 min. Remove the steel balls, brush the contents of the hardness pan onto a sieve of the fine-mesh size designated on the label, shake for 3 min on the mechanical sieve shaker, and weigh.

**Acceptance criteria:** The percentage of Barium Hydroxide Lime retained on the screen is NLT 75.0% and represents the hardness.

### • CARBON DIOXIDE ABSORBENCY

**Analysis:** Fill the lower transverse section of a U-shaped drying tube of about 15-mm internal diameter and 15-cm height with loosely packed glass wool. Place in one arm of the tube about 5 g of anhydrous calcium chloride, and weigh the tube and the contents. Into the other arm of the tube, place 9.5–10.5 g of Barium Hydroxide Lime, and again weigh. Insert stoppers in the open arms of the U-tube, and connect the side tube of the arm filled with Barium Hydroxide Lime to a calcium chloride drying tube, which in turn is connected to a suitable supply source of carbon dioxide. Pass the carbon dioxide through the U-tube at a rate of 75 mL/min for 20 min, timed. Disconnect the U-tube, cool to room temperature, remove the stoppers, and weigh.

**Acceptance criteria:** The increase in weight is NLT 19.0% of the weight of Barium Hydroxide Lime used for the test.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** If an indicator has been added, the name and color change of such indicator are stated on the container label. The container label indicates also the mesh size in terms of standard-mesh sieve sizes (see *Powder Fineness* (811)).

## Barium Sulfate

BaSO<sub>4</sub> 233.39  
Sulfuric acid, barium salt (1:1);  
Barium sulfate (1:1) [7727-43-7].

### DEFINITION

Barium Sulfate contains NLT 97.5% and NMT 100.5% of barium sulfate (BaSO<sub>4</sub>).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Sulfate** (191)  
Sample solution: Mix 0.5 g of Barium Sulfate with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter.  
Acceptance criteria: The filtrate, acidified with hydrochloric acid, meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Barium** (191)  
Sample solution: Dissolve a portion of the well-washed residue from *Identification test A* in 6 N acetic acid.  
Acceptance criteria: The solution meets the requirements.

### ASSAY

- **PROCEDURE**  
Sample: 0.58–0.62 g, weighed in a tared platinum crucible  
Analysis: Add 10 g of anhydrous sodium carbonate to the crucible, and mix by rotating the crucible. Fuse over



a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ( $\text{BaSO}_4$ ).

Acceptance criteria: 97.5%–100.5%

## IMPURITIES

Delete the following:

### • HEAVY METALS (231)

**Sample solution:** Boil 4.0 g with a mixture of 2 mL of glacial acetic acid and 48 mL of water for 10 min. Dilute with water to 50 mL, filter, and use 25 mL of the filtrate.

Acceptance criteria: NMT 10 ppm (Official 1-Jan-2018)

### • LIMIT OF SULFIDE

**Sample solution:** Transfer 10 g to a 500-mL conical flask. Add 100 mL of 0.3 N hydrochloric acid.

**Control solution:** 100 mL of 0.3 N hydrochloric acid containing 5 µg of sulfide in a 500-mL conical flask

**Analysis:** Cover the mouth of both conical flasks with a circle of filter paper that has been moistened at the area over the mouth of the flask with 0.15 mL of lead acetate TS, the paper being held in place with a string tied around the neck of the flask. Boil each mixture gently for 10 min, taking care to avoid spattering the paper.

Acceptance criteria: NMT 0.5 µg/g; any darkening of the paper by the *Sample solution* is not greater than that produced by the similarly treated *Control solution*.

### • LIMIT OF ACID-SOLUBLE SUBSTANCES

**Sample solution:** Cool the mixture obtained in the test for *Limit of Sulfide*, add water to restore approximately the original volume, and filter it through paper that previously has been washed with a mixture of 10 mL of 3 N hydrochloric acid and 90 mL of water, returning the first portions, if necessary, to obtain a clear filtrate.

**Analysis:** Evaporate 50 mL of the filtrate on a steam bath to dryness, and add 2 drops of hydrochloric acid and 10 mL of hot water. Filter again through acid-

washed paper, prepared as directed above. Wash the filter with 10 mL of hot water, and evaporate the combined filtrate and washings in a tared dish on a steam bath to dryness. Dry the residue at 105° for 1 h.

Acceptance criteria: NMT 0.3%; the residue weighs NMT 15 mg.

### • LIMIT OF SOLUBLE BARIUM SALTS

**Sample:** The residue obtained in the test for *Limit of Acid-Soluble Substances*

**Control:** 10 mL of water containing 0.5 mL of 2 N sulfuric acid and 50 µg of barium

**Analysis:** Treat the *Sample* with 10 mL of water, pass the solution through a filter previously washed with 100 mL of 0.3 N hydrochloric acid, and add 0.5 mL of 2 N sulfuric acid.

Acceptance criteria: NMT 0.001%; any turbidity formed in the *Sample* within 30 min is NMT that produced in the similarly treated *Control*.

## SPECIFIC TESTS

- **PH (791):** 3.5–10.0, in a 10% (w/w) aqueous suspension

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## Barium Sulfate Paste

### DEFINITION

Barium Sulfate Paste is a semisolid formulation of finely divided particles of Barium Sulfate in a suitable base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate ( $\text{BaSO}_4$ ). It may contain one or more suitable colors, flavors, suspending or dispersing agents, and preservatives.

### IDENTIFICATION

#### • A. IDENTIFICATION TESTS—GENERAL, Sulfate (191)

**Sample:** Ignite a quantity of Paste equivalent to 0.5 g of barium sulfate to constant weight.

**Analysis:** Mix 0.5 g of the ignited *Sample* with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter. Proceed as directed in the chapter.

Acceptance criteria: The filtrate, acidified with hydrochloric acid, meets the requirements.

#### • B. IDENTIFICATION TESTS—GENERAL, Barium (191)

**Sample solution:** Dissolve a portion of the well-washed residue from *Identification test A* in 6 N acetic acid.

Acceptance criteria: The solution meets the requirements.

### ASSAY

#### • PROCEDURE

**Sample:** Barium Sulfate Paste, equivalent to 0.60 g of barium sulfate, weighed in a tared platinum crucible

**Analysis:** Ignite the *Sample* over a low flame until any organic matter is thoroughly carbonized. Cool, cautiously add 0.5 mL of nitric acid and 0.5 mL of sulfuric acid, and continue the ignition over a low flame until the residue becomes gray in color, then ignite over the full heat of a blast burner. Allow the contents of the crucible to cool to room temperature.

[NOTE—If the specimen contains a silicate, such as bentonite, proceed as follows. Add 10 mL of water and 1 mL of sulfuric acid to the residue in the crucible, mix, and add 10 mL of hydrofluoric acid. Heat gently over a low flame until fumes of sulfur trioxide appear. Add 5 mL more of hydrofluoric acid, heat again over a low flame to the appearance of dense fumes, and continue heating until the sulfuric acid has been com-



pletely volatilized. Allow the contents of the crucible to cool.]

[NOTE—If the specimen does not contain a silicate, omit the treatment of the specimen with hydrofluoric and sulfuric acids.]

Add to the treated or untreated specimen in the platinum crucible 10 g of anhydrous sodium carbonate, fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ( $\text{BaSO}_4$ ).

Acceptance criteria: 90.0%–110.0%

## SPECIFIC TESTS

### • MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62)

For products labeled for oral administration only:

The total aerobic microbial count does not exceed  $10^2$  cfu/g. The total combined molds and yeasts count does not exceed  $10^1$  cfu/g. It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The total enterobacterial count does not exceed  $10^1$  cfu/g.

For products labeled for oral administration and rectal administration:

The total aerobic microbial count does not exceed  $10^2$  cfu/g. The total combined molds and yeasts count does not exceed  $10^1$  cfu/g. It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The total enterobacterial count does not exceed  $10^1$  cfu/g.

For products labeled for rectal administration only:

The total aerobic microbial count does not exceed  $10^3$  cfu/g. The total combined molds and yeasts count does not exceed  $10^2$  cfu/g. It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The total enterobacterial count does not exceed  $10^1$  cfu/g.

- **PH (791):** 3.0–10.0

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from freezing and from excessive heat.

## Barium Sulfate Suspension

### DEFINITION

Barium Sulfate Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate ( $\text{BaSO}_4$ ). It contains suitable dispersing and/or suspending agents so that when mixed as directed in the labeling, it yields a uniformly dispersed suspension. It may contain one or more suitable colors, flavors, fluidizing agents, and preservatives.

### IDENTIFICATION

#### • A. IDENTIFICATION TESTS—GENERAL, Sulfate (191)

**Sample:** Shake the Suspension, and transfer a volume equivalent to 0.5 g of barium sulfate to a suitable container. Ignite to constant weight.

**Analysis:** Mix 0.5 g of the ignited *Sample* with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter. Proceed as directed in the chapter.

**Acceptance criteria:** The filtrate, acidified with hydrochloric acid, meets the requirements.

#### • B. IDENTIFICATION TESTS—GENERAL, Barium (191)

**Sample solution:** Dissolve a portion of the well-washed residue from *Identification test A* in 6 N acetic acid.

**Acceptance criteria:** The solution meets the requirements.

### ASSAY

#### • PROCEDURE

**Sample:** A volume of Suspension, previously well shaken in its original container, equivalent to 0.60 g of barium sulfate, in a tared platinum crucible

**Analysis:** Ignite over a low flame until any organic matter is thoroughly carbonized. Cool, cautiously add 0.5 mL of nitric acid and 0.5 mL of sulfuric acid, and continue the ignition over a low flame until the residue becomes gray in color, then ignite over the full heat of a blast burner. Allow the contents of the crucible to cool to room temperature.

[NOTE—If the specimen contains a silicate, such as bentonite, proceed as follows. Add 10 mL of water and 1 mL of sulfuric acid to the residue in the crucible, mix, and add 10 mL of hydrofluoric acid. Heat gently over a low flame until fumes of sulfur trioxide appear. Add 5 mL more of hydrofluoric acid, heat again over a low flame to the appearance of dense fumes, and continue heating until the sulfuric acid has been completely volatilized. Allow the contents of the crucible to cool.]

[NOTE—If the specimen does not contain a silicate, omit the treatment of the specimen with hydrofluoric and sulfuric acids.]

Add to the treated or untreated specimen in the platinum crucible 10 g of anhydrous sodium carbonate, fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath



until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ( $\text{BaSO}_4$ ).

Acceptance criteria: 90.0%–110.0%

#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total bacterial count does not exceed  $10^2$  cfu/mL, the total combined molds and yeasts count does not exceed  $10^1$  cfu/mL, and it meets the requirements of the tests for absence of *Salmonella* species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.
- **PH** (791): 3.5–10.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.

### Barium Sulfate for Suspension

#### DEFINITION

Barium Sulfate for Suspension is a dry mixture of Barium Sulfate and one or more suitable dispersing and/or suspending agents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate ( $\text{BaSO}_4$ ). It may contain one or more suitable colors, flavors, fluidizing agents, and preservatives.

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Sulfate** (191)  
**Sample:** Ignite 1 g to constant weight.  
**Analysis:** Mix 0.5 g of the ignited *Sample* with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter.  
**Acceptance criteria:** The filtrate, acidified with hydrochloric acid, meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Barium** (191)  
**Sample solution:** Dissolve a portion of the well-washed residue from *Identification test A* in 6 N acetic acid.  
**Acceptance criteria:** The solution meets the requirements.

#### ASSAY

##### • PROCEDURE

**Sample:** Barium Sulfate for Suspension, equivalent to 0.60 g of barium sulfate, weighed in a tared platinum crucible

**Analysis:** Ignite over a low flame until any organic matter is thoroughly carbonized. Cool, cautiously add 0.5 mL of nitric acid and 0.5 mL of sulfuric acid, and continue the ignition over a low flame until the residue becomes gray in color, then ignite over the full heat of a blast burner. Allow the contents of the crucible to cool to room temperature.

[NOTE—If the specimen contains a silicate, such as bentonite, proceed as follows. Add 10 mL of water and 1 mL of sulfuric acid to the residue in the crucible, mix, and add 10 mL of hydrofluoric acid. Heat gently over a low flame until fumes of sulfur trioxide appear. Add 5 mL more of hydrofluoric acid, heat again over a low flame to the appearance of dense fumes, and continue heating until the sulfuric acid has been completely volatilized. Allow the contents of the crucible to cool.]

[NOTE—If the specimen does not contain a silicate, omit the treatment of the specimen with hydrofluoric and sulfuric acids.]

Add to the treated or untreated specimen in the platinum crucible 10 g of anhydrous sodium carbonate, fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so obtained, multiplied by 0.9213, represents the weight of barium sulfate ( $\text{BaSO}_4$ ).

Acceptance criteria: 90.0%–110.0%

#### SPECIFIC TESTS

- **LOSS ON DRYING** (731)  
**Analysis:** Dry at 105° for 4 h.  
**Acceptance criteria:** NMT 1.0%
- **PH** (791): 3.5–10.0, in a 60% (w/w) aqueous suspension, or constituted for its intended use as directed in the labeling



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

**Barium Sulfate Tablets****DEFINITION**

Barium Sulfate Tablets are flat-sided disks between 11.5 mm and 13.5 mm in diameter and contain NLT 90.0% and NMT 110.0% of the labeled amount of barium sulfate ( $\text{BaSO}_4$ ).

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Sulfate (191)**

**Sample:** A portion of powdered Tablets equivalent to 0.6 g of barium sulfate

**Analysis:** Mix the *Sample* with 2 g each of anhydrous sodium carbonate and anhydrous potassium carbonate, heat the mixture in a crucible until fusion is complete, treat the resulting fused mass with hot water, and filter. Proceed as directed in the chapter.

**Acceptance criteria:** The filtrate, acidified with hydrochloric acid, meets the requirements.

- **B. IDENTIFICATION TESTS—GENERAL, Barium (191)**

**Sample solution:** Dissolve a portion of the well-washed residue from *Identification* test A in 6 N acetic acid.

**Acceptance criteria:** The solution meets the requirements.

**ASSAY**

- **PROCEDURE**

**Sample:** A portion of powdered Tablets, equivalent to 0.6 g of barium sulfate, weighed in a tared platinum crucible

**Analysis:** Add 10 g of anhydrous sodium carbonate to the crucible, and mix by rotating the crucible. Fuse over a blast burner until a clear melt is obtained, and heat for an additional 30 min. Cool, place the crucible in a 400-mL beaker, add 250 mL of water, stir with a glass rod, and heat to dislodge the melt. Remove the crucible from the beaker, and wash with water, collecting the washings in the beaker. Rinse the inside of the crucible with 2 mL of 6 N acetic acid and then with water, again collecting the washings in the beaker, and continue heating and stirring until the melt is disintegrated. Cool the beaker in an ice bath until the precipitate settles, and decant the clear liquid through filter paper (Whatman No. 40, or equivalent), taking care to transfer as little precipitate as possible to the paper.

Wash twice by decantation as follows. Wash down the inside of the beaker with 10 mL of cold sodium carbonate solution (1 in 50), swirl the contents of the beaker, allow the precipitate to settle, and decant the supernatant through the same filter paper as before, transferring as little precipitate as possible to the paper. Place the beaker containing the bulk of the barium carbonate precipitate under the funnel, wash the filter paper with five 1-mL portions of 3 N hydrochloric acid, and wash the paper with water. [NOTE—The solution may be slightly hazy.]

Add 100 mL of water, 5.0 mL of hydrochloric acid, 10.0 mL of ammonium acetate solution (2 in 5), 25 mL of potassium dichromate solution (1 in 10), and 10.0 g of urea. Cover the beaker with a watch glass, and digest at 80°–85° for NLT 16 h. Filter while hot through a tared, fine-porosity, sintered-glass crucible, transferring all of the precipitate with the aid of a rubber-tipped stirring rod. Wash the precipitate with potassium dichromate solution (1 in 200), and finally with 20 mL of water. Dry at 105° for 2 h, cool, and weigh. The weight of the barium chromate so ob-

tained, multiplied by 0.9213, represents the weight of barium sulfate ( $\text{BaSO}_4$ ).

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **DISINTEGRATION (701):** NLT 10 min and NMT 30 min
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

**BCG Live****DEFINITION**

BCG Live (intravesical) for immunotherapy is a freeze-dried preparation of attenuated live bacteria derived from a culture of bacillus Calmette-Guérin (*Mycobacterium bovis* var. BCG) and is used intravesically in the treatment of carcinoma in situ and papilloma tumors of the urinary bladder. The bacteria are grown in a medium that does not contain substances known to cause toxic or allergic reactions in human beings or to cause the bacteria to become virulent for guinea pigs. The culture is harvested and formulated to contain one or more excipients. The freeze-dried preparation is reconstituted and further diluted aseptically with a sterile diluent for use. A reconstituted dose contains  $1.0\text{--}19.2 \times 10^8$  cfu. BCG Live does not contain a preservative.

**IDENTIFICATION**

- **A.** BCG Live is identified by microscopic examination of the bacilli in stained smears demonstrating their acid-fast property. Alternatively, validated molecular biology techniques may be used.

**SPECIFIC TESTS**

- **STERILITY TESTS (71):** It meets the requirements when tested as directed for *Direct Inoculation of the Culture Medium in Test for Sterility of the Product to Be Examined*.

- **GENERAL SAFETY**

(See *Biological Reactivity Tests, In Vivo (88)*, *Safety Tests—Biologicals*.)

**Sample solution:** 3.0 mL of the reconstituted product

**Analysis:** Guinea pigs are injected intraperitoneally with *Sample solution*.

**Acceptance criteria:** Meets the requirements

- **VIRULENT MYCOBACTERIA**

**Sample solution:** Reconstitute the freeze-dried BCG Live as per the manufacturer's instructions for human use with the diluent recommended by the manufacturer, and dilute aseptically with sterile BCG diluent to about 2 mg/mL.

**Analysis:** Randomly select NLT six guinea pigs of the same sex, each weighing 250–300 g. Inject each animal with a total of at least 4 mg of the *Sample solution* intramuscularly or subcutaneously in the rear left internal thigh, and observe them for a period of 6 weeks. Note the number of animals that survive at the end of the observation period, then sacrifice them. Perform autopsies of all animals postmortem to examine them for evidence of tuberculous infections, particularly at the popliteal and inguinal lymph nodes, liver, spleen, pancreas, and lungs, as well as at the injection site. If any abnormalities are found, perform a histological examination using standard and acid-fast staining techniques to detect acid-fast organisms.

**Acceptance criteria:** The product complies with the test if none of the animals show signs of tuberculosis and NMT one-third of the animals die during the observation period.



### • SKIN REACTIVITY

**Sample solutions:** Using the diluent and the *Sample solution* prepared as directed in *Virulent Mycobacteria*, further dilute aseptically by making three serial 10-fold dilutions.

**Analysis:** Randomly select two guinea pigs (male or female), each weighing 250–300 g. Inject 0.1 mL of each of the four *Sample solutions* intradermally at different sites on the back of each animal. After 4 weeks, the animals are shaved so that the injection sites and any reactions are made clearly visible. The diameters of the reactions are measured, and the presence of necrosis or nodules are noted.

**Acceptance criteria:** The reaction for the largest dose is between 4 and 10 mm and the smallest dose induces a nodule less than or equal to 4 mm. Each animal gains weight during the observation period.

### • TUBERCULIN SENSITIVITY

**Tuberculin solution:** Use tuberculin, purified protein derivative, to prepare a solution containing 25 U.S. Tuberculin Units/0.1 mL. Dilute aseptically, if necessary, with sterile 0.9% sodium chloride solution.

**Analysis:** Use the same animals on which the *Skin Reactivity* test is performed. After the *Skin Reactivity* test is completed, inject each animal intradermally on the back with 0.1 mL of the *Tuberculin solution*, and observe after 18–24 h.

**Acceptance criteria:** An erythematous reaction of NLT 10 mm in diameter is measured on each animal.

### • RESIDUAL MOISTURE:

NMT the limit approved for the particular product, determined by a suitable validated method. Limits vary in accordance with the method.

### • POTENCY:

Determine the number of viable Units/mL by viable count on solid medium using a method suitable for the product to be examined. Alternatively, a validated biochemical method may be used.

### • VIABILITY

**Samples:** NLT five containers of BCG Live before freeze-drying, and an equal number of containers after freeze-drying.

**Analysis:** Determine the potencies of BCG Live following the procedure described in *Potency*, except use the suspension before freeze-drying as is.

**Acceptance criteria:** The loss in viability due to freeze-drying is NMT 90%.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** BCG Live is sensitive to light and therefore must be preserved and stored in a glass container where it is protected from direct light at a temperature between 2° and 8°.
- **EXPIRATION DATE:** The product is stable for 3 years when stored between 2° and 8°.
- **LABELING:** Label it to indicate the dry weight of bacteria in a vial, cfu/dose, the storage conditions, the expiration date, and that it is not to be used after the expiration date given on the package. Label it to state that it should be protected from light and that it should be used immediately after reconstitution/dilution. Label it to indicate that it is "for intravesical use".

## BCG Vaccine

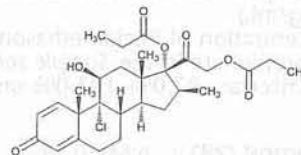
» BCG Vaccine conforms to the regulations of the FDA concerning biologics (see *Biologics* (1041)). It is a dried, living culture of the bacillus Calmette-Guérin strain of *Mycobacterium tuberculosis* var. *bovis*, grown in a suitable medium from

a seed strain of known history that has been maintained to preserve its capacity for conferring immunity. It contains an amount of viable bacteria such that inoculation, in the recommended dose, of tuberculin-negative persons results in an acceptable tuberculin conversion rate. It is free from other organisms, and contains a suitable stabilizer. It contains no antimicrobial agent. [NOTE—Use the Vaccine immediately after its constitution, and discard any unused portion after 2 hours.]

**Packaging and storage**—Preserve in hermetic containers, preferably of Type I glass, at a temperature between 2° and 8°.

**Expiration date**—The expiration date is not later than 6 months after date of issue, or not later than 1 year after date of issue if stored at a temperature below 5°.

## Beclomethasone Dipropionate



$C_{28}H_{37}ClO_7 \cdot H_2O$  539.07

$C_{28}H_{37}ClO_7$  521.04

Pregna-1,4-diene-3,20-dione, 9-chloro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (11 $\beta$ ,16 $\beta$ )-; 9-Chloro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [5534-09-8].

### DEFINITION

Beclomethasone Dipropionate is anhydrous or contains one molecule of water of hydration. It contains NLT 97.0% and NMT 103.0% of beclomethasone dipropionate ( $C_{28}H_{37}ClO_7$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile and water (60:40) such that the retention times for beclomethasone dipropionate and testosterone propionate are approximately 6 and 10 min, respectively.

**Internal standard solution:** 1.2 mg/mL of USP Testosterone Propionate RS in methanol

**Standard stock solution:** 1.4 mg/mL of USP Beclomethasone Dipropionate RS in methanol

**Standard solution:** 0.7 mg/mL of USP Beclomethasone Dipropionate RS and 0.6 mg/mL of USP Testosterone Propionate RS prepared as follows. Transfer 4.0 mL of *Standard stock solution* to a suitable vial, and add 4.0 mL of *Internal standard solution*.

**Sample stock solution:** 1.4 mg/mL of Beclomethasone Dipropionate in methanol

**Sample solution:** 0.7 mg/mL of Beclomethasone Dipropionate and 0.6 mg/mL of USP Testosterone Propionate RS prepared as follows. Transfer 4.0 mL of *Sample stock solution* to a suitable vial, and add 4.0 mL of *Internal standard solution*.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; packing L1

Injection volume: 5–25 µL

**System suitability**Sample: *Standard solution***Suitability requirements**

Relative standard deviation: NMT 3.0% for five replicate injections

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of beclomethasone dipropionate ( $C_{28}H_{37}ClO_7$ ) in the portion of Beclomethasone

Dipropionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak height ratio of beclomethasone dipropionate to the internal standard from the *Sample solution*

$R_S$  = peak height ratio of beclomethasone dipropionate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Beclomethasone Dipropionate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Beclomethasone Dipropionate in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**SPECIFIC TESTS**

- **OPTICAL ROTATION, Specific Rotation** (781S)

Sample solution: 10 mg/mL in dioxane

Acceptance criteria: +88° to +94°

- **LOSS ON DRYING** (731)

Analysis: Dry a sample at 105° for 3 h.

Acceptance criteria: NMT 0.5% for the anhydrous form; 2.8%–3.8% for the monohydrate form

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
USP Beclomethasone Dipropionate RS  
USP Testosterone Propionate RS

## Beclomethasone Dipropionate Compounded Oral Solution

**DEFINITION**

Beclomethasone Dipropionate Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of beclomethasone dipropionate ( $C_{28}H_{37}ClO_7$ ).

Prepare Beclomethasone Dipropionate Compounded Oral Solution 0.5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Beclomethasone Dipropionate powder	50 mg
Corn Oil, NF, a sufficient quantity to make	100 mL

Pour the *Beclomethasone Dipropionate powder* into a suitable container. Wet the powder with a small amount of *Corn Oil* and triturate to make a smooth paste. Add the *Corn Oil* to make the contents pourable. Transfer contents step-

wise and quantitatively to a calibrated container using the *Corn Oil*. Add sufficient *Corn Oil* to bring to final volume. Place on a shaker until dissolved. [NOTE—May take up to 24 h to dissolve.]

**ASSAY**• **PROCEDURE**

Mobile phase: Acetonitrile and water (65:35)

**Standard stock solution:** 0.5 mg/mL of beclomethasone dipropionate prepared from USP Beclomethasone Dipropionate RS in ethanol. Sonicate and mix well.

**Standard solution:** 0.02 mg/mL of beclomethasone dipropionate prepared from *Standard stock solution* and ethanol

**Sample solution:** Transfer 1.0 mL of Oral Solution to a 25-mL volumetric flask, add approximately 20 mL of ethanol, vortex for 30 s, and warm under running water until dissolved. Dilute with ethanol to volume.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV-Vis 240 nm

Column: 2.0-mm × 10-cm; 2.5-µm packing L1

Column temperature: 35°

Flow rate: 0.35 mL/min

Injection volume: 5 µL

**System suitability**Sample: *Standard solution*

[NOTE—The retention time for beclomethasone dipropionate is about 3.2 min.]

**Suitability requirements**

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of beclomethasone dipropionate ( $C_{28}H_{37}ClO_7$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of beclomethasone dipropionate from the *Sample solution*

$r_S$  = peak response of beclomethasone dipropionate from the *Standard solution*

$C_S$  = concentration of USP Beclomethasone Dipropionate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of beclomethasone dipropionate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Package in tight, light-resistant, plastic containers. Store at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at controlled room temperature.
- **LABELING:** Label it to be well-shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** (11)  
USP Beclomethasone Dipropionate RS

## Belladonna Leaf

» Belladonna Leaf consists of the dried leaf and flowering or fruiting top of *Atropa belladonna* L. or of its variety *acuminata* Royle ex Lindl. (Fam.



Solanaceae). Belladonna Leaf yields not less than 0.35 percent of the alkaloids of belladonna leaf.

**Packaging and storage**—Preserve in well-closed containers and avoid long exposure to direct sunlight. Preserve powdered Belladonna Leaf in light-resistant containers.

**USP Reference standards** (11)—

USP Atropine Sulfate RS

USP Homatropine Hydrobromide RS

USP Scopolamine Hydrobromide RS

**Botanic characteristics**—

**Belladonna Leaf**—Usually partly matted together, crumpled or broken leaves, together with some smaller stems and a number of flowers and fruits. The leaves are thin and brittle, mostly light green to moderate olive-green. The lamina is mostly from 5 to 25 cm in length and from 4 to 12 cm in width and possesses an ovate-lanceolate to broadly ovate outline, an acute to acuminate apex, an entire margin, an acute to somewhat decurrent base and slightly hairy surface, the hairs being more abundant along the veins; when broken transversely, it shows numerous light-colored dots (crystal cells) visible with a lens. The petiole is slender and usually up to 4 cm in length. The flowers possess a campanulate corolla with 5 small, reflexed lobes, purplish to yellowish purple, becoming faded to brown or dusky yellow or yellow, a green, 5-lobed calyx, 5 epipetalous stamens, and a superior, bilocular ovary with numerous ovules. The fruit is subglobular, dark yellow to yellowish brown to dusky red or black, up to about 12 mm in width and sometimes subtended by the persistent calyx and containing numerous flattened, somewhat reniform seeds, the latter up to about 2 mm in width. The stems are more or less flattened and hollow and finely hairy when young.

**Histology**—**Leaf**: The epidermis of the lamina possesses wavy anticlinal walls and a distinctly striated cuticle. Stomata are more numerous in the lower epidermis and are surrounded by 3 or 4 neighboring cells, one of which is smaller than the others. The nonglandular hairs are uniseriate and up to 6-celled. Short club-shaped glandular hairs with a 1-celled stalk and multicellular head and long glandular hairs with a uniseriate stalk and unicellular head occur on both epidermises. The mesophyll consists of a single layer of palisade parenchyma beneath which occurs spongy parenchyma, the latter with scattered cells filled with microcrystals. The midrib contains an arc of bicollateral bundles, collenchyma beneath upper epidermis, and scattered parenchyma cells with microcrystals. **Stem**: The stem shows an epidermis with striated cuticle and few hairs, a distinct endodermis, small strands of long, thin-walled, slightly lignified pericyclic fibers, and a circle of bicollateral bundles. The parenchyma of the cortex and pith is interspersed with crystal cells. **Flower**: The calyx possesses numerous glandular hairs with uniseriate stalks and 1- to 3-celled glandular heads. The corolla shows a papillose inner epidermis and an outer epidermis with glandular hairs similar to those of the calyx. The pollen grains, when mounted in chloral hydrate solution, are subspherical, about 40  $\mu$ m in diameter, tricolpate, having 3 germinal furrows and rows of pits between the ridges on the exine. **Fruit**: The epicarp exhibits polygonal epidermal cells with a striated cuticle and stomata. The mesocarp consists of large pulp cells some of which contain rosette aggregate crystals of calcium oxalate. **Seed**: The seed is characterized by an epidermis of large, wavy-walled cells with prominent ridges over the anticlinal walls.

**Powdered Belladonna Leaf**—Light olive-brown to moderate olive-green in color. The following are among the elements of identification: the separate microcrystals, the dark gray crystal cells, the cuticular striping of the epidermal cells, the vessels with ellipsoidal bordered pits, the fibers of the stem,

and occasional hairs and pollen grains. Rosette aggregates of calcium oxalate and fragments of the seed occur when the drug contains belladonna fruits. Examine Belladonna Leaf for hairs having a papillose cuticle and for raphides of calcium oxalate: their presence indicates adulteration.

**Acid-insoluble ash** (561): not more than 3.0%.

**Belladonna stems**—The proportion of belladonna stems over 10 mm in diameter does not exceed 3.0%.

**Assay**—

*pH 9.5 Phosphate buffer, Internal standard solution, Standard preparation, Extraction blank, Standard curve, Chromatographic system, and System suitability*—Proceed as directed in the Assay under Belladonna Extract.

**Assay preparation**—Moisten 10 g, previously reduced to a moderately coarse powder and accurately weighed, with a mixture of 8 mL of ammonium hydroxide, 10 mL of alcohol, and 20 mL of ether, and extract the alkaloids by either of the methods given in the following two paragraphs. If necessary, reduce the volume of the extract to 100 mL by evaporation on a steam bath.

*I*—Place the moistened drug in a continuous-extraction thimble, and allow maceration to proceed overnight, then extract with ether for 3 hours, or longer if necessary to effect complete extraction.

*II*—Place the moistened drug in a small percolator, and allow maceration to proceed overnight. Percolate slowly with a mixture of 3 volumes of ether and 1 volume of chloroform. Continue the percolation until the residue from 3 to 4 mL of percolate last passed, when dissolved in dilute sulfuric acid (1 in 70) and treated with mercuric iodide TS, shows not more than a faint turbidity.

Transfer the extract to a separator with the aid of ether. Extract with five 15-mL portions of dilute sulfuric acid (1 in 70), filtering each portion drawn off into a 100-mL volumetric flask. Wash the filter with dilute sulfuric acid (1 in 70), and collect the washings in the flask. Add dilute sulfuric acid (1 in 70) to volume, and mix. Dilute 20.0 mL of the resulting solution with the same dilute acid to 100.0 mL.

Pipet 10 mL of this solution into a 60-mL separator. To the separator add 1.0 mL of *Internal standard solution*, then add 15 mL of chloroform, shake vigorously, allow the layers to separate, and discard the chloroform layer. (If emulsions are formed, a *mixed solvent* consisting of chloroform-isopropyl alcohol (10:3) may be substituted for chloroform throughout the extraction procedure.) Add another 15 mL of chloroform, and extract again, discarding the chloroform phase. Add 15 mL of *pH 9.5 Phosphate buffer* and sufficient 1 N sodium hydroxide to yield a final pH between 9.0 and 9.5. Add 15 mL of chloroform, shake vigorously, and allow the layers to separate. Filter the organic phase through 10 g of anhydrous sodium sulfate (see *Sodium Sulfate, Anhydrous*, in the section *Reagent Specifications*), previously washed with chloroform and supported in a funnel with a small pledget of glass wool, into a suitable container. Extract again with two 15-mL portions of chloroform, again collecting the clarified organic phase. Wash the sodium sulfate and the tip of the funnel with 5 mL of chloroform. Evaporate the combined organic phases under reduced pressure, at a temperature below 45°, add 1 mL of chloroform, and mix to dissolve the alkaloids, taking care to wet the sides of the container.

**Procedure**—Record from the *Standard curves* the quantities, in mg, of atropine and scopolamine in the weight of the specimen taken. Proceed as directed for *Procedure* in the Assay under Belladonna Extract, through the next-to-the-last sentence. Add the quantity, in mg, of atropine and scopolamine, and multiply by 50 to obtain the weight, in mg, of alkaloids in the portion of Belladonna Leaf taken.



## Belladonna Extract

» Belladonna Extract contains, in each 100 g, not less than 1.15 g and not more than 1.35 g of the alkaloids of belladonna leaf.

### PILULAR BELLADONNA EXTRACT

Prepare the extract by percolating 1000 g of Belladonna Leaf, using a mixture of 3 volumes of alcohol and 1 volume of water as the menstruum. Macerate the drug for 16 hours, and then percolate it at a moderate rate. Evaporate the percolate under reduced pressure and at a temperature not exceeding 60° to a pilular consistency, and adjust the remaining extract, after assaying, by dilution with liquid glucose so that the finished Extract will contain, in each 100 g, 1.25 g of the alkaloids of belladonna leaf.

### POWDERED BELLADONNA EXTRACT

Prepare the extract by percolating 1000 g of Belladonna Leaf, using alcohol as the menstruum. Macerate the drug for 16 hours, and then percolate it slowly. Evaporate the percolate under reduced pressure and at a temperature not exceeding 60° to a soft extract, add 50 g of dry starch, and continue the evaporation, at the same temperature, until the product is dry. Powder the residue. The extract may be deprived of its fat by treating either the soft extract first obtained, or the dry and powdered extract, as directed under *Extracts* (see *Pharmaceutical Dosage Forms* (1151)). Assay the powdered residue, and add sufficient starch, previously dried at 100°, to obtain a finished Extract containing 1.25 g of the alkaloids of belladonna leaf in each 100 g. Mix the powders, and pass the Extract through a fine sieve.

**Packaging and storage**—Preserve in tight containers, at a temperature not exceeding 30°.

### USP Reference standards (11)—

USP Atropine Sulfate RS  
USP Homatropine Hydrobromide RS  
USP Scopolamine Hydrobromide RS

### Assay—

**pH 9.5 Phosphate buffer**—Dissolve 34.8 g of dibasic potassium phosphate in 900 mL of water, and adjust to a pH of 9.5, determined electrometrically, by the addition of 3 N hydrochloric acid or sodium hydroxide, with mixing.

**Internal standard solution**—Dissolve about 40 mg of USP Homatropine Hydrobromide RS, accurately weighed, in about 25 mL of dilute sulfuric acid (1 in 350) in a 50-mL volumetric flask, add the same dilute acid to volume, and mix. Prepare fresh on the day of use.

**Standard preparation**—Dissolve about 10 mg of USP Scopolamine Hydrobromide RS, accurately weighed, in about 5 mL of dilute sulfuric acid (1 in 350) in a 10-mL volumetric flask, add the same dilute acid to volume, and mix (*Solution A*). Dissolve about 20 mg of USP Atropine Sulfate RS, accurately weighed, in about 25 mL of dilute sulfuric acid (1 in 350) in a 50-mL volumetric flask, add 2.0 mL of *Solution A*, and mix. Add dilute sulfuric acid (1 in 350) to volume, and mix. Prepare fresh on the day of use.

**Extraction blank**—Place about 10 mL of dilute sulfuric acid (1 in 350) in a 60-mL separator. Proceed as directed under *Assay preparation*, beginning with "then add 15 mL of chloroform." The blank chromatogram contains no significant interferences at the locus of atropine, scopolamine, or homatropine.

**Assay preparation**—Weigh accurately about 0.5 g of Extract, transfer to a 125-mL conical flask, and add 40 mL of dilute sulfuric acid (1 in 350). Heat to a temperature not above 45°, and stir to hasten solution. Filter the solution through filter paper into a 100-mL volumetric flask. Wash the flask and the filter with two 20-mL portions of warmed dilute sulfuric acid (1 in 350), and collect the washings in the 100-mL volumetric flask. Add dilute sulfuric acid (1 in 350) to volume, and mix.

Pipet 10 mL of this solution into a 60-mL separator. To the separator add 1.0 mL of *Internal standard solution*, then add 15 mL of chloroform, shake vigorously, allow the layers to separate, and discard the chloroform layer. (If emulsions are formed, a *mixed solvent* consisting of chloroform and isopropyl alcohol (10:3) may be substituted for chloroform throughout the extraction procedure.) Add another 15 mL of chloroform, and extract again, discarding the chloroform phase. Add 15 mL of pH 9.5 Phosphate buffer and sufficient 1 N sodium hydroxide to yield a final pH between 9.0 and 9.5. Add 15 mL of chloroform, shake vigorously, and allow the layers to separate. Filter the organic phase through 10 g of anhydrous sodium sulfate (see *Suitability for alkaloid assays* under *Sodium Sulfate, Anhydrous*, in the section *Reagents, Indicators, and Solutions*), previously washed with chloroform and supported in a funnel with a small pledget of glass wool, into a suitable container. Extract again with two 15-mL portions of chloroform, again collecting the clarified organic phase. Wash the sodium sulfate and the tip of the funnel with 5 mL of chloroform. Evaporate the combined organic phases under reduced pressure, at a temperature below 45°, add 1 mL of chloroform, and mix to dissolve the alkaloids, taking care to wet the sides of the container.

**Standard curve**—Prepare three *Standard solutions* as follows. Pipet into three separate 60-mL separators 1.0-, 2.0-, and 3.0-mL portions, respectively, of *Standard preparation*, and add 9.0, 8.0, and 7.0 mL, respectively, of dilute sulfuric acid (1 in 350). Proceed as directed under *Assay preparation*, beginning with "add 1.0 mL of *Internal standard solution*."

**Chromatographic system**—Under typical condition, the instrument contains a 1.2-m × 4-mm glass column packed with 3% G3 on S1AB. The column may be cured and conditioned as specified under *Gas Chromatography* (see *Chromatography* (621)). The column is maintained at a temperature of about 215°, and the injection port and detector block at about 240°, and dry helium is used as a carrier gas at a flow rate of about 65 mL per minute.

**System suitability**—Chromatograph six to ten injections of the *Assay preparation*, and record peak areas as directed for *Procedure*. The analytical system is suitable for conducting this assay if the relative standard deviation for the ratio,  $R_A$ , calculated by the formula:

$$100 \times (\text{standard deviation} / \text{mean ratio})$$

does not exceed 2.0%; the resolution,  $R$ , between  $a_H$  and  $a_A$  is not less than 3; and the tailing factor (the sum of the distances from peak center to the leading edge and to the trailing edge divided by twice the distance from peak center to the leading edge), measured at 5% of the peak height of  $a_A$ , does not exceed 2.0.

**Procedure**—Inject a portion (about 5  $\mu$ L) of each *Standard solution* into a suitable gas chromatograph equipped with a flame-ionization detector. Measure the areas,  $a_A$ ,  $a_H$ , and  $a_S$ , of the atropine, homatropine, and scopolamine peaks, re-



spectively, in each chromatogram, and calculate the ratios  $A_A$  and  $A_S$  by the formulas:

$$a_A / a_H \text{ and } a_S / a_H.$$

Plot the *Standard curves* of the values of  $R_A$  and  $R_S$  against the amounts, in mg, of atropine and scopolamine in the solutions. (The ratio of the molecular weight of atropine to that of anhydrous atropine sulfate is 0.8551, and the ratio of the molecular weight of scopolamine to that of anhydrous scopolamine hydrobromide is 0.7894.) Inject a portion of the *Assay preparation* into the chromatograph, obtain the chromatogram area ratios, measure the peak areas, and calculate the area ratios, as with the *Standard solutions*. Record from the *Standard curve* the quantities, in mg, of atropine and scopolamine in the volume of specimen taken. Add the quantity, in mg, of atropine and scopolamine, and multiply by 10 to obtain the weight, in mg, of alkaloids in the portion of Extract taken.

### Belladonna Extract Tablets

» Belladonna Extract Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of the alkaloids of belladonna leaf.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Atropine Sulfate RS  
USP Homatropine Hydrobromide RS  
USP Scopolamine Hydrobromide RS

**Identification**—Macerate a quantity of powdered Tablets, equivalent to about 5 mg of the alkaloids of belladonna extract, with 20 mL of water, and transfer to a separator. Render the solution alkaline with 6 N ammonium hydroxide, and extract the alkaloids with 50 mL of chloroform. Filter the chloroform layer, divide it into two equal portions, and evaporate to dryness: the residue responds to the following tests.

**A:** To one portion of the dry residue add 2 drops of nitric acid, evaporate on a steam bath to dryness, and add a few drops of alcoholic potassium hydroxide TS: a violet color is produced.

**B:** Dissolve the other portion of the residue in 1 mL of dilute hydrochloric acid (1 in 120), and add gold chloride TS, dropwise with shaking, until a definite precipitate separates. Slowly heat until the precipitate dissolves, and allow the solution to cool: a lusterless precipitate is produced.

**Disintegration** (701): 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

pH 9.5 Phosphate buffer, *Internal standard solution*, *Standard preparation*, *Extraction blank*, *Standard curve*, *Chromatographic system*, and *System suitability*—Proceed as directed in the Assay under *Belladonna Extract*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 600 µg of atropine and scopolamine, to a 60-mL separator, add 10.0 mL of dilute sulfuric acid (1 in 350), and sonicate to dissolve as much as possible of the specimen. Proceed as directed for *Assay preparation* in the Assay under *Belladonna Leaf*, beginning with "add 1.0 mL of *Internal standard solution*."

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Belladonna Extract*. Record from the *Standard curves*

the quantities, in mg, of atropine and scopolamine in the weight of specimen taken.

### Belladonna Tincture

» Belladonna Tincture yields, from each 100 mL, not less than 27 mg and not more than 33 mg of the alkaloids of belladonna leaf.

Belladonna Leaf, in moderately

coarse powder .....	100 g
To make about .....	1000 mL

Prepare a tincture by *Process P* as modified for assayed *Tinctures* (see *Pharmaceutical Dosage Forms* (1151)), using a mixture of 3 volumes of alcohol and 1 volume of water as the menstruum. Finally adjust the Tincture to contain, in each 100 mL, 30 mg of the alkaloids of belladonna leaf.

**Packaging and storage**—Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

#### USP Reference standards (11)—

USP Atropine Sulfate RS  
USP Homatropine Hydrobromide RS  
USP Scopolamine Hydrobromide RS

**Alcohol Determination, Method II** (611): between 65.0% and 70.0% of  $C_2H_5OH$ , determined by the gas-liquid chromatographic procedure, acetone being used as the internal standard.

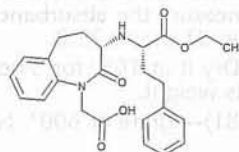
#### Assay—

pH 9.5 Phosphate buffer, *Internal standard solution*, *Standard preparation*, *Extraction blank*, *Standard curve*, *Chromatographic system*, and *System suitability*—Proceed as directed in the Assay under *Belladonna Extract*.

**Assay preparation**—Proceed with Tincture as directed in the Assay under *Belladonna Leaf*, but pipet 2 mL of Tincture (in place of "10 mL of this solution") into a 60-mL separator containing 10 mL of dilute sulfuric acid (1 in 350).

**Procedure**—Proceed as directed in the Assay under *Belladonna Leaf*. Record from the *Standard curve* the quantities, in mg, of atropine and scopolamine in the specimen. Add the quantity, in mg, of atropine and scopolamine, and multiply by 50 to obtain the weight, in mg, of alkaloids per 100 mL.

### Benazepril Hydrochloride



$C_{24}H_{28}N_2O_5 \cdot HCl$  460.95

1*H*-1-Benzazepine-1-acetic acid, 3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-, monohydrochloride, [5-( $R^*$ ,  $R^*$ )]-



(3S)-3-[[[(1S)-1-Carboxy-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-acetic acid, 3-ethyl ester, monohydrochloride [86541-74-4].

» Benazepril Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $C_{24}H_{28}N_2O_5 \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, and store at a temperature below 30°, preferably between 15° and 30°.

**USP Reference standards** (11)—

USP Benazepril Hydrochloride RS

USP Benazepril Related Compound A RS

(3R)-3-[[[(1R)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-acetic acid, monohydrochloride.

$C_{24}H_{28}N_2O_5 \cdot HCl$  460.95

USP Benazepril Related Compound B RS

(3S)-3-[[[(1R)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-acetic acid, monohydrochloride.

$C_{24}H_{28}N_2O_5 \cdot HCl$  460.95

USP Benazepril Related Compound C RS

3-(1-Carboxy-3-phenyl-1(S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid.

$C_{22}H_{24}N_2O_5$  396.44

USP Benazepril Related Compound D RS

(3-(1-Ethoxycarbonyl-3-cyclohexyl-1(S)-propyl)amino)-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid, monohydrochloride.

$C_{24}H_{34}N_2O_5 \cdot HCl$  467.00

USP Benazepril Related Compound E RS

3-Amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid.

USP Benazepril Related Compound F RS

tert-Butyl-3-amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid.

USP Benazepril Related Compound G RS

(3-(1-Ethoxycarbonyl-3-phenyl-1(S)-propyl)amino)-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid ethyl ester.

**Identification**—

A: *Infrared Absorption* (197M).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

C: It responds to the test for *Chloride* (191).

**Absorbance of solution**—The absorbance of a 1 in 100 solution of it in methanol, determined in a 1-cm cell at 420 nm, is not more than 0.015, methanol being used as the blank.

**Absorptivity**—

*Test preparation*—Dissolve an accurately weighed quantity of Benazepril Hydrochloride in methanol, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.025 mg per mL.

*Procedure*—Proceed as directed under *Ultraviolet-Visible Spectroscopy* (857), and measure the absorbance at 238 nm: the absorptivity is between 21.0 and 23.2.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 1.5% of its weight.

**Residue on ignition** (281)—Ignite at 600°. Not more than 0.1% residue is found.

**Delete the following:**

• **Heavy metals, Method II** (231): 0.001%. • (Official 1-Jan-2018)

**Related compounds**—

TEST 1 (FOR BENAZEPRIL RELATED COMPOUND A)—

*pH 6.0 Phosphate buffer*—Dissolve 9.66 g of monobasic potassium phosphate and 2.68 g of dibasic sodium phosphate, heptahydrate in about 900 mL of water, and dilute with water to 1000 mL.

*Mobile phase*—Prepare a filtered and degassed mixture of *pH 6.0 Phosphate buffer* and methanol (80:20). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Resolution solution*—Dissolve accurately weighed quantities of USP Benazepril Hydrochloride RS and USP Benazepril Related Compound A RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having known concentrations of about 1.0 mg per mL and 0.005 mg per mL, respectively.

*Standard stock solution*—Dissolve an accurately weighed quantity of USP Benazepril Related Compound A RS in *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

*Standard solution*—Dilute a suitable portion of *Standard stock solution*, accurately measured, with *Mobile phase* to obtain a solution having a known concentration of about 5 µg per mL.

*Dilute standard solution*—Dilute a suitable portion of *Standard stock solution*, accurately measured, with *Mobile phase* to obtain a solution having a known concentration of about 1 µg per mL.

*Test solution*—Transfer about 50 mg of Benazepril Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.0-mm × 10-cm column that contains packing L41. The flow rate is about 0.9 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between benazepril hydrochloride and benazepril related compound A is not less than 2.0. Chromatograph the *Dilute standard solution*, and record the peak responses as directed for *Procedure*: the signal-to-noise ratio is not less than 10:1. Chromatograph the *Standard solution*: the relative standard deviation for replicate injections determined from the benazepril related compound A peak is not more than 10%.

*Procedure*—Separately inject equal volumes (about 50 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the area for the benazepril related compound A peak. Calculate the percentage of benazepril related compound A in the portion of Benazepril Hydrochloride taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Benazepril Related Compound A RS in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Benazepril Hydrochloride in the *Test solution*;  $r_U$  is the peak response for benazepril related compound A obtained from the *Test solution*; and  $r_S$  is the peak response for benazepril related compound A obtained from the *Standard solution*: The limit of benazepril related compound A is given in the table below.



Benazepril Related Compound	Relative Retention Time	Limit (%)
A <sup>1</sup>	2.3	0.1

<sup>1</sup>[(3R)-3-[(1R)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-acetic acid, monohydrochloride

TEST 2 (FOR BENAZEPRIL RELATED COMPOUNDS B, C, D, E, F, AND G)—

*Tetrabutylammonium bromide solution, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the Assay.

*Standard solution*—Dissolve accurately weighed quantities of USP Benazepril Hydrochloride RS, USP Benazepril Related Compound B RS, USP Benazepril Related Compound C RS, USP Benazepril Related Compound D RS, USP Benazepril Related Compound E RS, USP Benazepril Related Compound F RS, and USP Benazepril Related Compound G RS in *Mobile phase* to obtain a solution having known concentrations of about 1 µg of USP Benazepril Hydrochloride RS per mL and 10 µg of each related compound per mL.

*Test solution*—Transfer about 50 mg of Benazepril Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Procedure*—Separately inject equal volumes (about 25 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Calculate the percentage of benazepril related compounds in the portion of Benazepril Hydrochloride taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of the relevant USP Reference Standard in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of benazepril hydrochloride in the *Test solution*;  $r_U$  is the peak response for the relevant benazepril related compound obtained from the *Test solution*; and  $r_S$  is the peak response for the relevant benazepril related compound obtained from the *Standard solution* (see Table 1 for values).

Table 1

Benazepril Related Compound	Relative Retention Time	Limit (%)
E <sup>1</sup>	0.4	0.2
F <sup>2</sup>	0.5	0.2
C <sup>3</sup>	0.6	0.3
B <sup>4</sup>	1.5	0.5
D <sup>5</sup>	1.7	0.2
G <sup>6</sup>	2.0	0.2

<sup>1</sup>3-Amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid

<sup>2</sup>4-Butyl-3-amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid

<sup>3</sup>3-(1-Carboxy-3-phenyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid

<sup>4</sup>Mixture of diastereoisomers (3-(1-ethoxycarbonyl-3-phenyl-(1R)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine)-1-acetic acid and (3-(1-ethoxycarbonyl-3-phenyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3R)-benzazepine)-1-acetic acid

<sup>5</sup>3-(1-Ethoxycarbonyl-3-cyclohexyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine)-1-acetic acid monohydrochloride

<sup>6</sup>3-(1-Ethoxycarbonyl-3-phenyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine)-1-acetic acid ethyl ester

In addition to not exceeding the limits for benazepril related compounds in Table 1, not more than 0.1% of any other single impurity is found; [NOTE—For calculating any other single unspecified impurity,  $C_S$  is the concentration of the USP Benazepril Hydrochloride RS in the *Standard solution*,] and not more than 2.0% of total impurities (excluding benazepril related compound A from Test 1) is found.

## Assay—

*Tetrabutylammonium bromide solution*—Dissolve 0.81 g of tetrabutylammonium bromide in 360 mL of water containing 0.2 mL of glacial acetic acid.

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and *Tetrabutylammonium bromide solution* (64:36). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve accurately weighed quantities of USP Benazepril Hydrochloride RS and USP Benazepril Related Compound B RS in *Mobile phase* to obtain a solution having known concentrations of about 0.4 mg of each per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Benazepril Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

*Assay preparation*—Transfer about 10.0 mL of the *Test solution* (from either Test 1 or Test 2), prepared as directed in the test for *Related compounds*, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 3-cm guard column that contains packing L1 connected to a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between benazepril hydrochloride and benazepril related compound B is not less than 1.7; and the relative standard deviation for replicate injections determined from benazepril hydrochloride and benazepril related compound B is not more than 2.0% for each.

*Procedure*—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the quantity, in mg, of  $C_{24}H_{28}N_2O_5 \cdot HCl$  in the portion of Benazepril Hydrochloride taken by the formula:

$$250C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Benazepril Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Benazepril Hydrochloride Tablets

» Benazepril Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—When more than one *Dissolution* test is given, the labeling states the test used only if Test 1 is not used.

### USP Reference standards (11)—

USP Benazepril Hydrochloride RS

USP Benazepril Related Compound B RS

(3S) 3-[(1R) 1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-acetic acid, monohydrochloride.

$C_{24}H_{28}N_2O_5 \cdot HCl$  460.95

USP Benazepril Related Compound C RS

3-(1-Carboxy-3-phenyl-(1S)-propyl)amino-2,3,4,5-tetrahydro-2-oxo-1H-1-(3S)-benzazepine-1-acetic acid.

$C_{22}H_{24}N_2O_5$  396.44



**Identification—**

**A: Thin-Layer Chromatographic Identification Test (201)—**

**Test solution**—Finely powder not fewer than 20 Tablets, and transfer an accurately weighed portion of the powder, equivalent to about 50 mg of benazepril hydrochloride, to a 50-mL volumetric flask. Add about 30 mL of methanol, and shake by mechanical means for 15 minutes. Dilute with methanol to volume, mix, and centrifuge. Pass an aliquot of the supernatant through a suitable filter, discarding the first 6 mL of the filtrate.

**Application volume:** 20  $\mu$ L.

**Developing solvent system:** a mixture of ethyl acetate, methanol, and ammonium hydroxide (80:20:15).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution (711)—**

**TEST 1—**

**Medium:** water, 500 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

Determine the amount of  $C_{24}H_{28}N_2O_5 \cdot HCl$  dissolved by employing the following method.

*Tetrabutylammonium bromide solution, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Benazepril Hydrochloride*.

**Procedure**—Inject about 60  $\mu$ L, or an amount of a filtered portion of the solution under test, equivalent to about 1.2  $\mu$ g of benazepril, into the chromatograph. The amount of benazepril injected should not exceed 1.5  $\mu$ g. Record the chromatogram, and measure the responses for the major peaks. Determine the quantity, in mg, of  $C_{24}H_{28}N_2O_5 \cdot HCl$  dissolved in comparison with a *Standard solution* having a known concentration of USP Benazepril Hydrochloride RS in the same *Medium* and similarly chromatographed.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{24}H_{28}N_2O_5 \cdot HCl$  is dissolved in 30 minutes.

**TEST 2**—If the product complies with this test, the labeling indicates that the product meets USP *Dissolution Test 2*.

**Medium, Apparatus, and Procedure**—Proceed as directed for *Test 1*.

**Time:** 45 minutes.

**Tolerances**—Not less than 70% (Q) of the labeled amount of  $C_{24}H_{28}N_2O_5 \cdot HCl$  is dissolved in 45 minutes.

**Uniformity of dosage units (905):** meet the requirements.

**PROCEDURE FOR CONTENT UNIFORMITY—**

*Tetrabutylammonium bromide solution, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay*.

**Test preparation**—Transfer 1 Tablet to a suitable volumetric flask, add a volume of *Mobile phase* equivalent to about 50% of the volume of the flask, sonicate for 5 minutes, and then shake by mechanical means for not less than 10 minutes. Dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a final concentration of about 0.2 mg per mL, mix, and pass a portion of the solution through a suitable filter, discarding the first 6 mL of the filtrate.

**Procedure**—Proceed as directed in the *Assay*, except to inject the *Test preparation* instead of the *Assay preparation*. Calculate the quantity, in mg, of benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ) in the Tablet taken by the formula:

$$VDC(r_U / r_S)$$

in which *V* is the volume, in mL, of the initial flask used to prepare the *Test preparation*; *D* is the dilution factor in sub-

sequent dilutions of *V*, if necessary, to prepare the *Test preparation*; *C* is the concentration, in mg per mL, of USP Benazepril Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the benazepril hydrochloride peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively.

**Related compounds—**

*Tetrabutylammonium bromide solution, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Benazepril Related Compound C RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.006 mg per mL.

**Test solution**—Use the *Assay preparation*.

**Procedure**—Separately inject equal volumes (about 80  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses of the peaks for benazepril related compound C. Calculate the percentage of benazepril related compound C in the portion of Tablets taken by the formula:

$$100(C_S / C_T)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP Benazepril Related Compound C RS in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of benazepril hydrochloride in the *Test solution*; and  $r_U$  and  $r_S$  are the peak responses for benazepril related compound C obtained from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% of benazepril related compound C is found. Calculate the percentage of each impurity (other than benazepril related compound C) in the portion of Tablets taken by the formula:

$$100(r_i / r_S)$$

in which  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_S$  is the sum of the responses of all the peaks (including benazepril related compound C): not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found, the results for all impurities (excluding benazepril related compound C) being added.

**Assay—**

*Tetrabutylammonium bromide solution, Mobile phase, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Benazepril Hydrochloride*.

**Assay preparation**—Finely powder not fewer than 20 Tablets, and transfer an accurately weighed portion of the powder, equivalent to about 50 mg of benazepril hydrochloride, to a 250-mL volumetric flask. Add about 150 mL of *Mobile phase*, and shake by mechanical means for 30 minutes. Dilute with *Mobile phase* to volume, mix, and centrifuge. Pass an aliquot of the supernatant through a suitable filter, discarding the first 6 mL of the filtrate.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for all the peaks. Calculate the quantity, in mg, of benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ) in the portion of Tablets taken by the formula:

$$250C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Benazepril Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the benazepril hydrochloride peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Benazepril Hydrochloride Compounded Oral Suspension, Veterinary

### DEFINITION

Benazepril Hydrochloride Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ).

Prepare Benazepril Hydrochloride Compounded Oral Suspension, Veterinary, 5 mg/mL, as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Benazepril Hydrochloride powder	500 mg
Vehicle: a 1:1 mixture of Ora-Plus <sup>a</sup> and Ora-Sweet <sup>a</sup> , a sufficient quantity to make	100 mL

<sup>a</sup> Perrigo Pharmaceuticals, Allegan, MI.

Pour the *Benazepril Hydrochloride powder* into a suitable container. Wet the powder with a small amount of *Vehicle*, and triturate to make a smooth paste. Add the *Vehicle* to make the contents pourable. Transfer the contents, stepwise and quantitatively, to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

### ASSAY

#### PROCEDURE

**Solution A:** 25 mM sodium phosphate adjusted with phosphoric acid to a pH of 3.0. Pass through a nylon filter of 0.45- $\mu$ m pore size.

**Mobile phase:** Acetonitrile and *Solution A* (40:60)

**Diluent:** Water adjusted with phosphoric acid to a pH of 3.0

**Standard stock solution:** 5 mg/mL of USP Benazepril Hydrochloride RS in *Diluent*. Sonicate for 3 min. Mix well, and store at 2°–8°.

**Standard solution:** 0.01 mg/mL of benazepril hydrochloride prepared with *Standard stock solution* and *Diluent*. Centrifuge for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

**Sample solution:** Shake thoroughly each bottle of Oral Suspension, Veterinary. Transfer 2.0 mL of the Oral Suspension, Veterinary into a 1-L volumetric flask, and dilute with *Diluent* to volume. Mix well. Centrifuge for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Temperatures**

**Column:** 30°

**Autosampler:** 5°

**Flow rate:** 1.2 mL/min

**Injection volume:** 25  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for benazepril hydrochloride is about 6.5 min.]

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benazepril hydrochloride ( $C_{24}H_{28}N_2O_5 \cdot HCl$ ) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of benazepril hydrochloride from the *Sample solution*

$r_s$  = peak response of benazepril hydrochloride from the *Standard solution*

$C_s$  = concentration of benazepril hydrochloride in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of benazepril hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

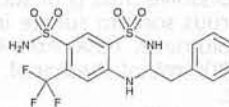
### SPECIFIC TESTS

- **pH (791):** 3.8–4.8

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be well-shaken before use, and to state the *Beyond-Use Date*. Label it to state that it is for veterinary use only.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8° or at controlled room temperature
- **USP REFERENCE STANDARDS (11)**  
USP Benazepril Hydrochloride RS

## Bendroflumethiazide



$C_{15}H_{14}F_3N_3O_4S_2$  421.41  
2H-1,2,4-Benzothiadiazine-7-sulfonamide, 3,4-dihydro-3-(phenylmethyl)-6-(trifluoromethyl)-, 1,1-dioxide, ( $\pm$ ); ( $\pm$ )-3-Benzyl-3,4-dihydro-6-(trifluoromethyl)-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [73-48-3].

### DEFINITION

Bendroflumethiazide contains NLT 98.0% and NMT 102.0% of  $C_{15}H_{14}F_3N_3O_4S_2$ , calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

**Sample:** Previously dried over silica gel for 4 h

**Acceptance criteria:** Meets the requirements

- **B. ULTRAVIOLET ABSORPTION (197U)**

**Analytical wavelength:** 271 nm

**Sample solution:** 10  $\mu$ g/mL in methanol

**Acceptance criteria:** Absorptivities, calculated on the anhydrous basis, do not differ by more than 4.0%.

- **C.**

**Sample solution:** Mix 5 mL of dilute hydrochloric acid (50% v/v) with 20 mg of Bendroflumethiazide, boil gently for 1 min, and cool in an ice bath.

**Analysis:** To the *Sample solution* add, in succession, 0.5 mL of sodium nitrite solution (1 mg/mL), 0.5 mL of ammonium sulfamate solution (5 mg/mL), and 0.5 mL of N-(1-naphthyl)ethylenediamine dihydrochloride solution (1 mg/mL).



Acceptance criteria: A deep red color is produced.

## ASSAY

### PROCEDURE

**Sample:** 190 mg of Bendroflumethiazide

**Analysis:** Dissolve the *Sample* in 80 mL of pyridine in a tall-form, 250-mL beaker in a well-ventilated hood. Add 3 drops of a saturated solution of azo violet in methanol, cover the beaker, and gently bubble nitrogen through the solution for 5 min, being careful to avoid any contact between the solution and the cover. Raise the nitrogen delivery tube above the solution surface and, maintaining a gentle flushing with nitrogen and stirring with a magnetic or mechanical stirring device, add 0.1 N sodium methoxide VS from a 10-mL buret inserted through an opening in the cover. Titrate to a blue endpoint, approaching the endpoint at a rate of 1 or 2 drop/s. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N sodium methoxide is equivalent to 21.07 mg of  $C_{15}H_{14}F_3N_3O_4S_2$ .

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

## IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

Delete the following:

- **HEAVY METALS**, Method II (231): 20 ppm (Official 1-Jan-2018)

### SELENIUM (291)

**Sample:** 100 mg of Bendroflumethiazide and 100 mg of magnesium oxide

Acceptance criteria: The absorbance from the *Test solution* is NMT one-half that from the *Standard solution* (NMT 30 ppm).

### LIMIT OF 2,4-DISULFAMYL-5-TRIFLUOROMETHYLANILINE

[NOTE—Use low-actinic glassware for the *Standard solution* and the *Sample solution*.]

**Mobile phase:** Dissolve 5.62 g of sodium chloride and 1.97 g of anhydrous sodium sulfate in 1000 mL of water in a 2-L volumetric flask. Add 4.0 mL of glacial acetic acid and 800 mL of methanol, and dilute with water to volume.

**Standard solution:** 0.75 µg/mL of USP 2,4-Disulfamyl-5-trifluoromethylaniline RS in methanol

**Sample solution:** 50 µg/mL of Bendroflumethiazide in methanol

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm × 30-cm; packing L11

**Column temperature:** 35° ± 5°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Resolution:** NLT 1.4 between the methanol and 2,4-disulfamyl-5-trifluoromethylaniline peaks

**Relative standard deviation:** NMT 3.0% for five replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 2,4-disulfamyl-5-trifluoromethylaniline in the portion of Bendroflumethiazide taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP 2,4-Disulfamyl-5-trifluoromethylaniline RS in the *Standard solution* (µg/mL)

$C_u$  = concentration of Bendroflumethiazide in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 1.5%

## SPECIFIC TESTS

- **WATER DETERMINATION**, Method I (921): NMT 0.5%

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

### USP REFERENCE STANDARDS (11)

USP Bendroflumethiazide RS

USP 2,4-Disulfamyl-5-trifluoromethylaniline RS

$C_{15}H_{14}F_3N_3O_4S_2$  319.29

## Bendroflumethiazide Tablets

### DEFINITION

Bendroflumethiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bendroflumethiazide ( $C_{15}H_{14}F_3N_3O_4S_2$ ).

### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

## ASSAY

### PROCEDURE

[NOTE—Use low-actinic glassware for the *Sample solution* and the *Standard solution*.]

**Mobile phase:** Dissolve 5.62 g of sodium chloride and 1.97 g of anhydrous sodium sulfate in 1000 mL of water in a 2-L volumetric flask. Add 4.0 mL of glacial acetic acid and 800 mL of methanol, and dilute with water to volume.

**Standard solution:** 50 µg/mL of USP Bendroflumethiazide RS in methanol

**Sample solution:** Nominally equivalent to 50 µg/mL from finely powdered Tablets (NLT 20). Initially add methanol (70% of the volume of the flask) and sonicate for 15 min with occasional shaking. Dilute further with methanol to the required concentration, and centrifuge for 15 min.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.6-mm × 30-cm; packing L11

**Temperature:** 35 ± 5°

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.0% for five replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{15}H_{14}F_3N_3O_4S_2$  in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Bendroflumethiazide RS in the *Standard solution* (µg/mL)



$C_u$  = nominal concentration of bendroflumethiazide in the *Sample solution* ( $\mu\text{g/mL}$ )

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

[NOTE—Protect solutions from light throughout this test.]

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Detector: UV 271 nm

Sample solution: Sample per *Dissolution* (711).

Standard solution: Prepare a stock solution of USP Bendroflumethiazide RS in an appropriate organic solvent, and dilute this solution with *Medium* to obtain a final concentration similar to the one expected in the *Sample solution*.

Analysis: Determine the amount of  $\text{C}_{15}\text{H}_{14}\text{F}_3\text{N}_3\text{O}_4\text{S}_2$  dissolved by using UV absorption on filtered portions of the *Sample solution*, suitably diluted with water, if necessary, in comparison with a *Standard solution* having a known concentration of USP Bendroflumethiazide RS.

Tolerances: NLT 75% (Q) of the labeled amount of  $\text{C}_{15}\text{H}_{14}\text{F}_3\text{N}_3\text{O}_4\text{S}_2$  is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE:

Preserve in tight containers.

### • USP REFERENCE STANDARDS (11)

USP Bendroflumethiazide RS

chloric acid is equivalent to 34.49 mg of benoxinate hydrochloride ( $\text{C}_{17}\text{H}_{28}\text{N}_2\text{O}_3 \cdot \text{HCl}$ ).

Acceptance criteria: 98.5%–101.5% on the dried basis

## IMPURITIES

### • RESIDUE ON IGNITION (281):

NMT 0.2%

### • ORDINARY IMPURITIES (466)

Standard solution: Methanol

Sample solution: Methanol

Eluant: A mixture of chloroform, cyclohexane, and diethylamine (5:4:1)

Visualization: 12

## SPECIFIC TESTS

### • PH (791)

Sample solution: 10 mg/mL

Acceptance criteria: 5.0–6.0

### • LOSS ON DRYING (731)

Analysis: Dry a sample at 105° for 3 h.

Acceptance criteria: NMT 1.0%

## ADDITIONAL REQUIREMENTS

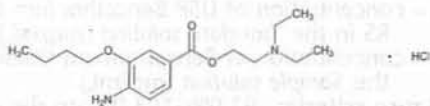
### • PACKAGING AND STORAGE:

Preserve in well-closed containers.

### • USP REFERENCE STANDARDS (11)

USP Benoxinate Hydrochloride RS

## Benoxinate Hydrochloride



$\text{C}_{17}\text{H}_{28}\text{N}_2\text{O}_3 \cdot \text{HCl}$  344.88

Benzoic acid, 4-amino-3-butoxy-, 2-(diethylamino)ethyl ester, monohydrochloride;

2-(Diethylamino)ethyl 4-amino-3-butoxybenzoate monohydrochloride [5987-82-6].

## DEFINITION

Benoxinate Hydrochloride contains NLT 98.5% and NMT 101.5% of benoxinate hydrochloride ( $\text{C}_{17}\text{H}_{28}\text{N}_2\text{O}_3 \cdot \text{HCl}$ ), calculated on the dried basis.

## IDENTIFICATION

### • A. INFRARED ABSORPTION (197K)

Sample: Previously dried

Acceptance criteria: Meets the requirements

### • B. ULTRAVIOLET ABSORPTION (197U)

Sample solution: 15  $\mu\text{g/mL}$  in water

Acceptance criteria: Meets the requirements

### • C. IDENTIFICATION TESTS—GENERAL, Chloride (191)

Sample solution: 10 mg/mL

Acceptance criteria: Meets the requirements

## ASSAY

### • PROCEDURE

Sample solution: Dissolve 250 mg of Benoxinate Hydrochloride in a mixture of 20 mL of glacial acetic acid and 20 mL of acetic anhydride.

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis: Titrate, perform a blank determination, and make any necessary correction. Each mL of 0.1 N per-

## Benoxinate Hydrochloride Ophthalmic Solution

## DEFINITION

Benoxinate Hydrochloride Ophthalmic Solution is a sterile solution of Benoxinate Hydrochloride in water. It contains NLT 95.0% and NMT 105.0% of the labeled amount of benoxinate hydrochloride ( $\text{C}_{17}\text{H}_{28}\text{N}_2\text{O}_3 \cdot \text{HCl}$ ).

## IDENTIFICATION

### • A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181)

Sample solution: Nominally 2 mg/mL of benoxinate hydrochloride, prepared as follows. Dilute a volume of solution, equivalent to 50 mg of benoxinate hydrochloride, with 0.01 N hydrochloric acid to 25 mL.

Analysis: Proceed as directed in the chapter, beginning with "Transfer the liquid to a separator".

Acceptance criteria: The solution meets the requirements.

## ASSAY

### • PROCEDURE

Standard solution: 400  $\mu\text{g/mL}$  of USP Benoxinate Hydrochloride RS in 0.1 N hydrochloric acid

Sample solution: Nominally 400  $\mu\text{g/mL}$  of benoxinate hydrochloride, prepared as follows. Transfer a volume of Ophthalmic Solution, equivalent to 20 mg of benoxinate hydrochloride, to a separator containing 15 mL of water. Add 1 mL of ammonium hydroxide, and extract with five 20-mL portions of ether. Wash the combined ether extracts with 10 mL of water, extract the water washing with 10 mL of ether, and add this ether extract to the main extract. Extract the ether solution with three 5-mL portions of 0.1 N hydrochloric acid, collect the acid extracts in a 50-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.

### Instrumental conditions

Mode: UV

Analytical wavelength: About 308 nm

Cell: 1 cm

Blank: 0.1 N hydrochloric acid

### Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*  
Transfer 5.0 mL of each sample to separate 200-mL volumetric flasks. Dilute the contents of each flask with



water to volume. Concomitantly determine the absorbances of the solutions, using the *Blank* to set the instrument.

Calculate the percentage of the labeled amount of benoxinate hydrochloride ( $C_{17}H_{28}N_2O_3 \cdot HCl$ ) in each mL of the Ophthalmic Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- $A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of USP Benoxinate Hydrochloride RS in the *Standard solution* (µg/mL)  
 $C_U$  = nominal concentration of benoxinate hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

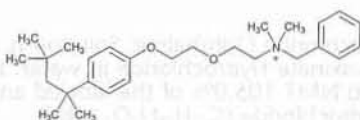
### SPECIFIC TESTS

- **STERILITY TESTS** (71): Meets the requirements
- **PH** (791): 3.0–6.0

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Benoxinate Hydrochloride RS

## Benzethonium Chloride



$C_{27}H_{42}ClNO_2$  448.08

Benzenemethanaminium, *N,N*-dimethyl-*N*-[2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]-, chloride; Benzyltrimethyl[2-[2-[*p*-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethyl]ammonium chloride [121-54-0].

### DEFINITION

Benzethonium Chloride contains NLT 97.0% and NMT 103.0% of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ), calculated on the dried basis.

### IDENTIFICATION

- **A.**  
*Sample solution:* 10 mg/mL  
*Analysis:* Add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS to 1 mL of the *Sample solution*.  
*Acceptance criteria:* A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.
- **B. INFRARED ABSORPTION** (197K)
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** Dilute 20 mL of triethylamine with water to 1000 mL, and adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Acetonitrile and *Buffer* (42:58)

**Diluent:** Acetonitrile and water (42:58)

**System suitability solution:** 0.15 mg/mL each of USP Benzethonium Chloride RS and USP Methylbenzethonium Chloride RS in *Diluent*

**Standard solution:** 0.15 mg/mL of USP Benzethonium Chloride RS in *Diluent*

**Sample solution:** 0.15 mg/mL of Benzethonium Chloride in *Diluent*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 225 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L7

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

**Run time:** 1.5 times the retention time of the methylbenzethonium peak

### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for benzethonium and methylbenzethonium are 0.7 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 7.0 between the benzethonium and methylbenzethonium peaks

**Tailing factor:** NMT 2.0 for the benzethonium peak

**Relative standard deviation:** NMT 1.0% for the benzethonium peak

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ) in the portion of Benzethonium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of benzethonium from the *Sample solution*

$r_S$  = peak response of benzethonium from the *Standard solution*

$C_S$  = concentration of USP Benzethonium Chloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Benzethonium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–103.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** (741): 158°–163°, the specimen having been dried previously
- **LOSS ON DRYING** (731)  
*Analysis:* Dry a sample at 105° for 4 h.  
*Acceptance criteria:* NMT 5.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Benzethonium Chloride RS  
USP Methylbenzethonium Chloride RS

## Benzethonium Chloride Concentrate

### DEFINITION

Benzethonium Chloride Concentrate contains NLT 94.0% and NMT 106.0% of the labeled amount of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ).

### IDENTIFICATION

#### • A.

**Sample:** Evaporate a volume of the Concentrate, equivalent to 200 mg of benzethonium chloride, on a steam bath.



**Analysis:** To the residue add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.

**Acceptance criteria:** A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

- **B.** **Sample:** Evaporate a volume of the Concentrate, equivalent to 200 mg of benzethonium chloride, on a steam bath.  
**Analysis:** To the residue add 0.1 g of potassium nitrate, and heat on a steam bath for 3 min. Cautiously dilute the solution with water to 10 mL, add 0.5 g of granulated zinc, and warm the mixture for 10 min. Cool. Add 0.2 g of sodium nitrite to 1 mL of the clear liquid, and add this mixture to 20 mg of naphthol dipotassium disulfonate or naphthol disodium disulfonate in 1 mL of ammonium hydroxide.  
**Acceptance criteria:** The solution turns orange-red, and a brown precipitate may be formed.

## ASSAY

### • PROCEDURE

**Sample solution:** Equivalent to 200 mg of benzethonium chloride from a volume of Concentrate, in a glass-stoppered flask

**Analysis:** Add 0.4 mL of bromophenol blue solution (1 in 2000), 10 mL of chloroform, and 1 mL of 1 N sodium hydroxide. Titrate with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 8.962 mg of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ).

**Acceptance criteria:** 94.0%–106.0% of the labeled amount of benzethonium chloride

## IMPURITIES

### • LIMIT OF NITRITES

**Sample:** One drop of Concentrate on a spot plate

**Analysis:** To the *Sample* add one drop each of glacial acetic acid, sulfanilic acid in acetic acid solution (1 in 100), and 1-naphthylamine-acetic acid solution (prepared by boiling 30 mg of 1-naphthylamine in 70 mL of water, decanting the colorless solution from the blue-violet residue, and mixing with 30 mL of glacial acetic acid).

**Acceptance criteria:** No red color develops in the resulting solution within 10 min.

## SPECIFIC TESTS

### • OXIDIZING SUBSTANCES

**Sample:** 5 mL

**Analysis:** To the *Sample* add 0.5 mL of potassium iodide TS and a few drops of 3 N hydrochloric acid.

**Acceptance criteria:** The solution does not acquire a yellow color.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at room temperature.
- **LABELING:** The label states that this article is not intended for direct administration to humans or animals.

## Benzethonium Chloride Topical Solution

### DEFINITION

Benzethonium Chloride Topical Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ).

## IDENTIFICATION

### • A.

**Sample solution:** Evaporate a volume of Topical Solution, equivalent to 200 mg of benzethonium chloride, on a steam bath.

**Analysis:** To the residue add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.

**Acceptance criteria:** A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

### • B.

**Sample solution:** Evaporate a volume of Topical Solution, equivalent to 200 mg of benzethonium chloride, on a steam bath.

**Analysis:** To the residue add 0.1 g of potassium nitrate, and heat on a steam bath for 3 min. Cautiously dilute the solution with water to 10 mL, add 0.5 g of granulated zinc, and warm the mixture for 10 min. Cool. Add 0.2 g of sodium nitrite to 1 mL of the clear liquid, and add this mixture to 20 mg of naphthol dipotassium disulfonate or naphthol disodium disulfonate in 1 mL of ammonium hydroxide.

**Acceptance criteria:** The solution turns orange-red, and a brown precipitate may be formed.

## ASSAY

### • PROCEDURE

**Sample solution:** Equivalent to 200 mg of benzethonium chloride from a volume of Topical Solution, in a glass-stoppered flask

**Analysis:** Add 0.4 mL of bromophenol blue solution (1 in 2000), 10 mL of chloroform, and 1 mL of 1 N sodium hydroxide. Titrate with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 8.962 mg of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ).

**Acceptance criteria:** 95.0%–105.0% of the labeled amount of benzethonium chloride

## IMPURITIES

### • ORGANIC IMPURITIES, LIMIT OF NITRITES

**Sample:** One drop of Topical Solution on a spot plate

**Analysis:** To the *Sample* add one drop each of glacial acetic acid, sulfanilic acid in acetic acid (1 in 100), and 1-naphthylamine-acetic acid solution (prepared by boiling 30 mg of 1-naphthylamine in 70 mL of water, decanting the colorless solution from the blue-violet residue, and mixing with 30 mL of glacial acetic acid).

**Acceptance criteria:** No red color develops in the resulting solution within 10 min.

## SPECIFIC TESTS

### • OXIDIZING SUBSTANCES

**Sample:** 5 mL

**Analysis:** To the *Sample* add 0.5 mL of potassium iodide TS and a few drops of 3 N hydrochloric acid.

**Acceptance criteria:** The solution does not acquire a yellow color.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

## Benzethonium Chloride Tincture

### DEFINITION

Benzethonium Chloride Tincture contains, in each 100 mL, NLT 190 mg and NMT 210 mg of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ).



Prepare Benzethonium Chloride Tincture 2 mg/mL as follows.

Benzethonium Chloride	2 g
Alcohol	685 mL
Acetone	100 mL
Purified Water, a sufficient quantity to make	1000 mL

Dissolve the *Benzethonium Chloride* in a mixture of *Alcohol* and *Acetone*. Add sufficient *Purified Water* to make 1000 mL. [NOTE—Benzethonium Chloride Tincture may be colored by the addition of any suitable color or combination of colors certified by the FDA for use in drugs.]

## IDENTIFICATION

### A. PROCEDURE

**Sample:** 50 mL

**Analysis:** To the residue obtained by evaporating the *Sample* on a steam bath, add 2 mL of alcohol, 0.5 mL of 2 N nitric acid, and 1 mL of silver nitrate TS.

**Acceptance criteria:** A white precipitate, which is insoluble in 2 N nitric acid but soluble in 6 N ammonium hydroxide, is formed.

### B. PROCEDURE

**Sample:** 50 mL

**Analysis:** Evaporate the *Sample* on a steam bath.

**Acceptance criteria:** The residue obtained forms precipitates with 2 N nitric acid and with mercuric chloride TS, both of which dissolve upon the addition of alcohol.

## ASSAY

### PROCEDURE

**Sample:** 50 mL

**Analysis:** Transfer the *Sample* to a 150-mL beaker, and add, with continuous stirring, 10 mL of 25 mg/mL of sodium tetraphenylboron solution. Cover, and allow to stand for 16 h. Decant the supernatant into a tared sintered-glass crucible, applying vacuum filtration. Suspend the precipitate in 20 mL of water. Transfer the precipitate to the crucible, washing well with water. Dry the precipitate and the crucible at 105° for 1 h, cool, and weigh. The weight of the precipitate so obtained, multiplied by 0.6122, represents its equivalent of benzethonium chloride ( $C_{27}H_{42}ClNO_2$ ).

**Acceptance criteria:** 190–210 mg

## OTHER COMPONENTS

### ALCOHOL AND ACETONE CONTENT

**Standard solution A (alcohol low standard solution):** Add 5.0 mL of methanol and 11.0 mL of dehydrated alcohol to a 100-mL volumetric flask, and dilute with water to volume.

**Standard solution B (alcohol high standard solution):** Add 5.0 mL of methanol and 14.0 mL of dehydrated alcohol to a 100-mL volumetric flask, and dilute with water to volume.

**Standard solution C (acetone low standard solution):** Add 5.0 mL of methanol and 1.7 mL of acetone to a 100-mL volumetric flask, and dilute with water to volume.

**Standard solution D (acetone high standard solution):** Add 5.0 mL of methanol and 2.2 mL of acetone to a 100-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 20 mL of Tincture to a 100-mL volumetric flask, add 5.0 mL of methanol as the internal standard, and dilute with water to volume.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 120-cm × 4-mm packed with a suitable type of support, such as 80- to 100-mesh S3

**Carrier gas:** Dry helium

**Temperature**

**Injector port:** 240°

**Detector block:** 240°

**Column:** 120°

**Flow rate:** 90 mL/min

**Injection size:** 0.8 µL

## Analysis

**Samples:** *Standard solutions A, B, C, and D, and Sample solution*

From the respective chromatograms obtained as described previously, calculate the ratios of peak areas for alcohol to internal standard and for acetone to internal standard.

Calculate the percentage of alcohol and of acetone in the portion of Tincture taken:

$$\text{Result} = [A(Y - Z) + B(Z - X)] / (Y - X)$$

**A** = percentage of alcohol, or of acetone, in *Standard solution A* and *Standard solution C*, respectively

**Y** = ratio of the alcohol peak areas, or the acetone peak areas, to the internal standard peak areas for *Standard solution B* and *Standard solution D*, respectively

**Z** = ratio of the alcohol peak areas, or the acetone peak areas, to the internal standard peak areas for the *Sample solution*

**B** = percentage of alcohol, or of acetone, in *Standard solution B* and *Standard solution D*, respectively

**X** = ratio of the alcohol peak areas, or the acetone peak areas, to the internal standard peak areas for *Standard solution A* and *Standard solution C*, respectively

## Acceptance criteria

**Alcohol ( $C_2H_5OH$ ):** 62.0%–68.0%

**Acetone ( $C_3H_6O$ ):** 9.0%–11.0%

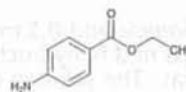
## SPECIFIC TESTS

**SPECIFIC GRAVITY (841):** 0.868–0.876

## ADDITIONAL REQUIREMENTS

**PACKAGING AND STORAGE:** Package in tight, light-resistant containers.

## Benzocaine



$C_9H_{11}NO_2$

165.19

Benzoic acid, 4-amino-, ethyl ester;

Ethyl *p*-aminobenzoate [94-09-7].

## DEFINITION

Benzocaine, dried over phosphorus pentoxide for 3 h, contains NLT 98.0% and NMT 102.0% of benzocaine ( $C_9H_{11}NO_2$ ).

## IDENTIFICATION

### A. INFRARED ABSORPTION (197K)

**Sample:** Previously dried over phosphorus pentoxide for 3 h

**Acceptance criteria:** Meets the requirements

**B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.



**ASSAY****PROCEDURE**

**Solution A:** Acetic acid, triethylamine, and water (20:1:980). The pH should be between 2.95 and 3.0 (adjust as needed).

**Mobile phase:** Methanol and *Solution A* (40:60)

**Standard solution:** 0.024 mg/mL of USP Benzocaine RS in *Mobile phase*

**Sample solution:** 0.024 mg/mL of Benzocaine in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 285 nm

**Column:** 2.0-mm × 15-cm; 5-μm packing L11

**Flow rate:** 0.2 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of benzocaine (C<sub>9</sub>H<sub>11</sub>NO<sub>2</sub>) in the portion of Benzocaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the previously dried basis

**IMPURITIES****RESIDUE ON IGNITION (281):** NMT 0.1%**Delete the following:****HEAVY METALS, Method II (231):** NMT 10 ppm (Official 1-

Jan-2018)

**CHLORIDE**

**Analysis:** To a solution of 200 mg in 5 mL of alcohol, previously acidified with a few drops of diluted nitric acid, add a few drops of silver nitrate TS.

**Acceptance criteria:** No turbidity is produced immediately.

**ORGANIC IMPURITIES**

**Solution A:** Add 1.0 mL of trifluoroacetic acid in 1 L of water.

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

**Diluent:** *Solution A* and *Solution B* (1:1)

**Standard stock solution:** 0.1 mg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-nitrobenzoate RS in *Diluent*. Sonicate for 2–5 min to dissolve before diluting to final volume.

**Standard solution:** 1 μg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-ni-

trobenzoate RS in *Diluent* from the *Standard stock solution*

**Sample solution:** 1 mg/mL of Benzocaine in *Diluent*. Sonicate for 2–5 min to assist in dissolution as needed before diluting to final volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 10 from any two peaks

**Relative standard deviation:** NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Benzocaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*

$r_S$  = peak response of corresponding reference standard from the *Standard solution*

$C_S$  = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-nitrobenzoate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Benzocaine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any other individual impurity from the *Sample solution*

$r_S$  = peak response of benzocaine from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 2*. Disregard peaks less than 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.29	0.10
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.10
Any other unspecified impurity	—	0.10
Total impurities	—	1.0

**SPECIFIC TESTS****LOSS ON DRYING (731)**

**Analysis:** Dry over phosphorus pentoxide for 3 h.

**Acceptance criteria:** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

**PACKAGING AND STORAGE:** Preserve in well-closed containers.



• **USP REFERENCE STANDARDS (11)**

USP Aminobenzoic Acid RS  
Benzoic acid, 4-amino.  
 $C_7H_7NO_2$  137.14  
USP Benzocaine RS  
USP Ethyl 4-nitrobenzoate RS  
Benzoic acid, 4-nitro-, ethyl ester.  
 $C_9H_9NO_4$  195.17

## Benzocaine Topical Aerosol

### DEFINITION

Benzocaine Topical Aerosol is a solution of Benzocaine in a pressurized container. It contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ).

### IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Solution A:** 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

**Diluent:** *Solution A* and *Solution B* (1:1)

**System suitability solution:** 1 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*

**Standard solution:** 0.1 mg/mL of USP Benzocaine RS in *Diluent*

**Sample composite:** Spray a portion of Topical Aerosol into a beaker or glass tube, and heat on a steam bath or a heating module at 100° for a few min to expel residual propellant. Use the resulting benzocaine solution.

**Sample solution:** Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a known quantity of benzocaine solution from a portion of *Sample composite* to an appropriate volumetric flask, dissolve, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm. For *Identification test A*, use a diode array detector in the range of 200–400 nm.

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 6 between aminobenzoic acid and benzocaine; NLT 6 between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ) in the portion of Topical Aerosol taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of benzocaine from the *Sample solution*

$r_s$  = peak response of benzocaine from the *Standard solution*

$C_s$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### IMPURITIES

#### ORGANIC IMPURITIES

**Solution A:** 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

**Diluent:** *Solution A* and *Solution B* (1:1)

**Standard solution:** 1 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*

**Sample composite:** Spray a portion of the Topical Aerosol into a beaker or a glass tube, and heat on a steam bath or a heating module at 100° for a few min to expel residual propellant. Use the resulting benzocaine solution.

**Sample solution:** Nominally 0.5 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer 50 mg of benzocaine from a portion of *Sample composite* to a 100-mL volumetric flask, dissolve, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—See Table 2 for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 6 between aminobenzoic acid and benzocaine; NLT 6 between benzocaine and ethyl 4-nitrobenzoate

**Relative standard deviation:** NMT 3% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Topical Aerosol taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*

$r_s$  = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Standard solution*



- $C_S$  = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual, unspecified impurity in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of any other individual, unspecified impurity from the *Sample solution*  
 $r_S$  = peak response of benzocaine from the *Standard solution*  
 $C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)  
**Acceptance criteria:** See Table 2. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.3	0.20
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.20
Any other individual, unspecified impurity	—	0.10
Total impurities	—	1.0

### SPECIFIC TESTS

- **TOPICAL AEROSOLS, Pressure Test (603):** Meets the requirements
- **LEAK RATE (604):** Meets the requirements
- **MINIMUM FILL (755):** Meets the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in pressurized containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS (11)**
  - USP Aminobenzoic Acid RS
  - Benzoic acid, 4-amino.
  - $C_7H_7NO_2$  137.14
  - USP Benzocaine RS
  - USP Ethyl 4-Nitrobenzoate RS
  - Benzoic acid, 4-nitro-, ethyl ester.
  - $C_9H_9NO_4$  195.17

## Benzocaine Cream

### DEFINITION

Benzocaine Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ) in a suitable cream base.

### IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Solution A:** 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

**Solution B:** Acetonitrile  
**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

**Diluent:** *Solution A* and *Solution B* (1:1)

**System suitability solution:** 1 µg/mL of USP Benzocaine RS and 2 µg/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-nitrobenzoate RS in *Diluent*

**Standard solution:** 0.1 mg/mL of USP Benzocaine RS in *Diluent*. Sonicate to dissolve, if necessary.

**Sample solution:** Nominally equivalent to 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Cream, nominally equivalent to 10 mg of benzocaine, into a 100-mL volumetric flask, and dissolve it in about 2% of the final volume of tetrahydrofuran. Dilute with *Diluent* to volume, and pass through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of filtrate.

### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm. For *Identification test A*, use a diode array detector in the range of 200–400 nm.

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 10 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of benzocaine from the *Sample solution*

$r_S$  = peak response of benzocaine from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

### IMPURITIES

#### ORGANIC IMPURITIES

**Solution A:** 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1 in the *Assay*.

**Diluent:** *Solution A* and *Solution B* (1:1)

**Standard solution:** 1 µg/mL of USP Benzocaine RS and 2 µg/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-nitrobenzoate RS in *Diluent*



**Sample solution:** Nominally equivalent to 1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Cream, nominally equivalent to 50 mg of benzocaine, into a volumetric flask, and dissolve in 10% of the final volume of tetrahydrofuran, with the aid of sonication as needed. Dilute with *Diluent* to volume, and pass through a suitable filter of 0.45- $\mu$ m pore size, discarding the first 2–3 mL of filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 10 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate

**Relative standard deviation:** NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*

$r_S$  = peak response of the corresponding Reference Standard from the *Standard solution*

$C_S$  = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-nitrobenzoate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any other individual unspecified impurity from the *Sample solution*

$r_S$  = peak response of benzocaine from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 2*. Disregard peaks less than 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.29	0.20
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.20
Any other individual unspecified impurity	—	0.10
Total impurities	—	1.0

#### SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and avoid prolonged exposure to temperatures exceeding 30°.

#### USP REFERENCE STANDARDS (11)

USP Aminobenzoic Acid RS

Benzoic acid, 4-amino.

$C_7H_7NO_2$  137.14

USP Benzocaine RS

USP Ethyl 4-nitrobenzoate RS

Benzoic acid, 4-nitro-, ethyl ester.

$C_9H_9NO_4$  195.17

## Benzocaine Gel

#### DEFINITION

Benzocaine Gel contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ).

#### IDENTIFICATION

- A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

**Solution A:** 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

**Diluent:** *Solution A* and *Solution B* (1:1)

**System suitability solution:** 1  $\mu$ g/mL of USP Benzocaine RS and 2  $\mu$ g/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-nitrobenzoate RS in *Diluent*

**Standard solution:** 0.1 mg/mL of USP Benzocaine RS in *Diluent*. Sonicate to dissolve, if necessary.

**Sample solution:** Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Gel, equivalent to 10 mg of benzocaine, into a 100-mL volumetric flask and dissolve it in *Diluent*. Pass through a suitable filter of 0.45- $\mu$ m pore size, discarding the first 2–3 mL of filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm. For *Identification test A*, use a diode array detector in the range of 200–400 nm.

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 10 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*



**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of benzocaine from the *Sample solution*

$r_S$  = peak response of benzocaine from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Solution A:** 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1 in the Assay.

**Diluent:** *Solution A* and *Solution B* (1:1)

**Standard solution:** 1 µg/mL of USP Benzocaine RS and 2 µg/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-nitrobenzoate RS in *Diluent*

**Sample solution:** Nominally 1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Gel, equivalent to 50 mg of benzocaine, into a 50-mL volumetric flask, and dissolve in *Diluent*. Pass through a suitable filter of 0.45-µm pore size, discarding the first 2–3 mL of filtrate.

#### Chromatographic system

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 10 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate

**Relative standard deviation:** NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*

$r_S$  = peak response of the corresponding Reference Standard from the *Standard solution*

$C_S$  = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-nitrobenzoate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any other individual unspecified impurity from the *Sample solution*

$r_S$  = peak response of benzocaine from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 2. Disregard peaks less than 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.29	0.20
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.20
Any other individual unspecified impurity	—	0.10
Total impurities	—	1.0

#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**

USP Aminobenzoic Acid RS

Benzoic acid, 4-amino.

$C_7H_7NO_2$  137.14

USP Benzocaine RS

USP Ethyl 4-nitrobenzoate RS

Benzoic acid, 4-nitro-, ethyl ester.

$C_9H_9NO_4$  195.17

## Benzocaine Lozenges

#### DEFINITION

Benzocaine Lozenges contain NLT 85.0% and NMT 120.0% of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ).

#### IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Buffer:** 1.0 M monobasic potassium phosphate, adjusted with phosphoric acid to a pH of 3.0

**Mobile phase:** Acetonitrile, water, and *Buffer* (250:700:50)

**Diluent A:** 0.1 N hydrochloric acid

**Diluent B:** Acetonitrile and water (1:1)

**Standard solution A:** 0.01 mg/mL of USP Benzocaine RS in *Diluent A*

**Standard solution B:** 0.01 mg/mL of USP Benzocaine RS in *Diluent B*

**Sample stock solution A:** Transfer the equivalent of 40 mg of benzocaine from powdered Lozenges (NLT 20) to a 200-mL volumetric flask. Add 150 mL of *Dilu-*



ent A, and stir for NLT 2 h. Dilute with *Diluent A* to volume.

**Sample stock solution B:** Transfer the equivalent of 40 mg of benzocaine from powdered Lozenges (NLT 20) to a 200-mL volumetric flask. Add 150 mL of *Diluent B*, and stir for NLT 30 min. Dilute with *Diluent B* to volume.

**Sample solution A:** Nominally 0.01 mg/mL of benzocaine in *Diluent A* from *Sample stock solution A*

**Sample solution B:** Nominally 0.01 mg/mL of benzocaine in *Diluent B* from *Sample stock solution B*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm. For *Identification* test A, use a diode array detector in the range of 200–400 nm.

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *Standard solution A* and *Standard solution B*

#### Suitability requirements

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*,

*Sample solution A*, and *Sample solution B*

Calculate the percentage of the total labeled amount of benzocaine ( $C_9H_{11}NO_2$ ) in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from *Sample solution A*

$r_S$  = peak response from *Standard solution A*

$C_S$  = concentration of USP Benzocaine RS in *Standard solution A* (mg/mL)

$C_U$  = nominal concentration of benzocaine in *Sample solution A* (mg/mL)

Calculate the percentage of free benzocaine ( $C_9H_{11}NO_2$ ) in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from *Sample solution B*

$r_S$  = peak response from *Standard solution B*

$C_S$  = concentration of USP Benzocaine RS in *Standard solution B* (mg/mL)

$C_U$  = nominal concentration of benzocaine in *Sample solution B* (mg/mL)

#### Acceptance criteria

**Total labeled amount of benzocaine:** 85.0%–120.0%

**Free benzocaine:** 85.0%–120.0%

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Solution A:** Dissolve 9.1 g of monobasic potassium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0.

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	60	40
10	45	55
10.1	60	40
13	60	40

**Diluent:** Acetonitrile and water (10:90)

**Standard stock solution:** 0.03 mg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP

Ethyl 4-Nitrobenzoate RS in *Diluent*. Sonicate for 2–5 min to dissolve before diluting to final volume.

**Standard solution:** 0.3 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent* from the *Standard stock solution*

**Sample solution:** Nominally 150 µg/mL of benzocaine in *Diluent* prepared as follows. Transfer 10 Lozenges to an appropriate volumetric flask to obtain a nominal benzocaine concentration of 0.15 mg/mL. Dissolve Lozenges in *Diluent* and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 100 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 10 between benzocaine and aminobenzoic acid; NLT 10 between ethyl 4-nitrobenzoate and benzocaine

**Relative standard deviation:** NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*

$r_S$  = peak response of the corresponding Reference Standard from the *Standard solution*

$C_S$  = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (µg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each unspecified degradation product from the *Sample solution*

$r_S$  = peak response of benzocaine from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (µg/mL)

**Acceptance criteria:** See *Table 2*. Disregard peaks less than 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.46	0.2
Benzocaine	1.00	—
Ethyl 4-nitrobenzoate	1.86	0.2
Any unspecified degradation product	—	0.1
Total degradation products	—	2.0



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
  - USP Aminobenzoic Acid RS
  - Benzoic acid, 4-amino.  
 $C_7H_7NO_2$  137.14
  - USP Benzocaine RS
  - USP Ethyl 4-Nitrobenzoate RS
  - Benzoic acid, 4-nitro-, ethyl ester.  
 $C_9H_9NO_4$  195.17

**Benzocaine Ointment****DEFINITION**

Benzocaine Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ) in a suitable ointment base.

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Solution A:** 0.1% Trifluoroacetic acid, prepared by adding 1.0 mL of trifluoroacetic acid to 1 L of water

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

**Diluent:** *Solution A* and *Solution B* (1:1)

**Standard solution:** 0.1 mg/mL of USP Benzocaine RS in *Diluent*. Sonication may be needed to aid in the dissolution.

**Sample solution:** Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows

**Ointments having water-soluble bases:** Transfer a portion of Ointment, equivalent to 10 mg of benzocaine, into a volumetric flask, and dissolve in *Diluent*.

**Ointments having water-insoluble bases:** Transfer a portion of Ointment, equivalent to 10 mg of benzocaine, into a volumetric flask, and dissolve in tetrahydrofuran, using about 2% of the final volume, then dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- $\mu$ m pore size, discarding the first 2–3 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm. For *Identification test B*, use a diode array detector in the range of 200–400 nm.

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 0.73%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of benzocaine from the *Sample solution*

$r_S$  = peak response of benzocaine from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**IMPURITIES**• **ORGANIC IMPURITIES**

**Solution A:** 0.1% Trifluoroacetic acid, prepared by adding 1.0 mL of trifluoroacetic acid to 1 L of water

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1 in the *Assay*.

**Diluent:** *Solution A* and *Solution B* (1:1)

**System suitability solution:** 0.2 mg/mL of USP Benzocaine RS and 0.01 mg/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-Nitrobenzoate RS in *Diluent*

**Standard solution:** 1  $\mu$ g/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*

**Sample solution:** Nominally 1 mg/mL of benzocaine in *Diluent* prepared as follows

**Ointments having water-soluble bases:** Transfer a portion of Ointment, equivalent to 50 mg of benzocaine, into a volumetric flask, and dissolve in *Diluent*.

**Ointments having water-insoluble bases:** Transfer a portion of Ointment, equivalent to 50 mg of benzocaine, into a volumetric flask, and dissolve in about 10% of final volume of tetrahydrofuran, then dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- $\mu$ m pore size, discarding the first 2–3 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 10 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*  
 $r_S$  = peak response of the corresponding Reference Standard from the *Standard solution*  
 $C_S$  = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response for any other individual unspecified impurity from the *Sample solution*  
 $r_S$  = peak response of benzocaine from the *Standard solution*  
 $C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.29	0.10
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.10
Any other individual unspecified impurity	—	0.10
Total impurities	—	1.0

## PERFORMANCE TESTS

- MINIMUM FILL (755):** Meets the requirements

## SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and store at room temperature at 15°–25°.
- USP REFERENCE STANDARDS (11)**
  - USP Aminobenzoic Acid RS  
Benzoic acid, 4-amino.  
 $C_7H_7NO_2$  137.14
  - USP Benzocaine RS  
USP Ethyl 4-Nitrobenzoate RS  
Benzoic acid, 4-nitro-, ethyl ester.  
 $C_9H_9NO_4$  195.17

## Benzocaine Otic Solution

### DEFINITION

Benzocaine Otic Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

- B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

## ASSAY

### PROCEDURE

**Solution A:** 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L  
**Solution B:** Acetonitrile  
**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

**Diluent:** *Solution A* and *Solution B* (1:1)

**System suitability solution:** 1 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*. Sonicate to dissolve, if necessary.

**Standard solution:** 0.1 mg/mL of USP Benzocaine RS in *Diluent*. Sonicate to dissolve, if necessary.

**Sample solution:** Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Otic Solution, equivalent to 10 mg of benzocaine, into a 100-mL volumetric flask and dissolve it in *Diluent*.

### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm. For Identification test B, use a diode array detector in the range of 200–400 nm.

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 6 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ) in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of benzocaine from the *Sample solution*

$r_S$  = peak response of benzocaine from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

## IMPURITIES

### ORGANIC IMPURITIES

**Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution:** 1 µg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Ni-



trobenzoate RS in *Diluent*. Sonicate to dissolve, if necessary.

**Sample solution:** Nominally 0.5 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer a portion of Otic Solution, equivalent to 50 mg of benzocaine, into a 100-mL volumetric flask, dissolve, and dilute with *Diluent* to volume.

#### System suitability

**Sample:** *Standard solution*

[NOTE—See Table 2 for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 6 between aminobenzoic acid and benzocaine, and between benzocaine and ethyl 4-nitrobenzoate

**Relative standard deviation:** NMT 3.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*  
 $r_S$  = peak response of the corresponding Reference Standard from the *Standard solution*  
 $C_S$  = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Otic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of any individual unspecified degradation product from the *Sample solution*  
 $r_S$  = peak response of benzocaine from the *Standard solution*  
 $C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 2. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.3	0.20
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.20
Any individual unspecified degradation product	—	0.20
Total impurities	—	1.0

#### SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli* and for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The total aerobic microbial count is less than  $10^2$  cfu/mL.

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- USP REFERENCE STANDARDS (11)**
  - USP Aminobenzoic Acid RS
  - Benzoic acid, 4-amino.  
 $C_7H_7NO_2$  137.14
  - USP Benzocaine RS
  - USP Ethyl 4-Nitrobenzoate RS
  - Benzoic acid, 4-nitro-, ethyl ester.  
 $C_9H_9NO_4$  195.17

### Benzocaine Topical Solution

#### DEFINITION

Benzocaine Topical Solution is a solution of Benzocaine in a suitable solvent. It contains NLT 90.0% and NMT 110.0% of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ). It contains a suitable antimicrobial agent.

#### IDENTIFICATION

- A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

**Solution A:** 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

**Diluent:** *Solution A* and *Solution B* (1:1)

**System suitability solution:** 1 µg/mL of USP Benzocaine RS and 2 µg/mL each of USP Aminobenzoic Acid RS and USP Ethyl 4-Nitrobenzoate RS in *Diluent*

**Standard solution:** 0.1 mg/mL of USP Benzocaine RS in *Diluent*

**Sample solution:** Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer 10 mg of benzocaine from a portion of Topical Solution to a 100-mL volumetric flask, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size as needed, discarding the first 2–3 mL of filtrate.

#### Chromatographic system

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm. For *Identification* test A, use a diode array detector in the range of 200–400 nm.

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for aminobenzoic acid, benzocaine, and ethyl 4-nitrobenzoate are 0.3, 1.0, and 2.1, respectively.]



**Suitability requirements**

**Resolution:** NLT 6 between aminobenzoic acid and benzocaine; and between benzocaine and ethyl 4-nitrobenzoate, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of benzocaine from the *Sample solution*

$r_S$  = peak response of benzocaine from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**IMPURITIES**• **ORGANIC IMPURITIES**

**Solution A:** 0.1% Trifluoroacetic acid, prepared by diluting 1.0 mL of trifluoroacetic acid with water to 1 L

**Solution B:** Acetonitrile

**Mobile phase:** See Table 2.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	85	15
34	55	45
35	85	15
38	85	15

**Diluent:** *Solution A* and *Solution B* (1:1)

**Standard solution:** 1 µg/mL of USP Benzocaine RS and 2 µg/mL each USP Aminobenzoic Acid RS and USP Ethyl 4-Nitrobenzoate RS in *Diluent*

**Sample solution:** Nominally 1 mg/mL of benzocaine in *Diluent* prepared as follows. Transfer 50 mg of benzocaine from a portion of Topical Solution to a volumetric flask and dissolve with aid of sonication as needed, then dilute with *Diluent* to volume. Pass through a suitable filter of 0.45-µm pore size as needed, discarding the first 2–3 mL of filtrate.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—See Table 3 for relative retention times.]

**Suitability requirements**

**Resolution:** NLT 6 between aminobenzoic acid and benzocaine; and between benzocaine and ethyl 4-nitrobenzoate

**Relative standard deviation:** NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*

$r_S$  = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Standard solution*

$C_S$  = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual unspecified impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any other individual unspecified impurity from the *Sample solution*

$r_S$  = peak response of benzocaine from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 3. Disregard peaks less than 0.05%.

**Table 3**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.27	0.20
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.5	0.20
Any other individual unspecified impurity	—	0.10
Total impurities	—	1.0

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, and avoid prolonged exposure to temperatures exceeding 30°.

• **USP REFERENCE STANDARDS** <11>

USP Aminobenzoic Acid RS

Benzoic acid, 4-amino.

$C_7H_7NO_2$  137.14

USP Benzocaine RS

USP Ethyl 4-Nitrobenzoate RS

Benzoic acid, 4-nitro-, ethyl ester.

$C_9H_9NO_4$  195.17



## Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Aerosol

### DEFINITION

Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Aerosol is Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution packaged in a pressurized container with a suitable inert propellant. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ).

### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Diluent:** Methanol and water (60:40)

**Mobile phase:** Methanol, water, and 0.25 M sodium 1-heptanesulfonate (500:500:20)

**Standard solution:** Transfer 140 mg of USP Benzocaine RS to a 100-mL volumetric flask. Swirl with the aid of 25 mL of methanol, and transfer 140/ mg of USP Butamben RS to the same volumetric flask with the aid of 25 mL of water, *f* being the ratio of the labeled amount, as a percentage, of butamben to the labeled amount, as a percentage, of benzocaine in the Topical Aerosol. Transfer 140/*f* mg of USP Tetracaine Hydrochloride RS into the same volumetric flask with the aid of 25 mL of water, *f* being the ratio of the labeled amount, as a percentage, of tetracaine hydrochloride to the labeled amount, as a percentage, of benzocaine in the Topical Aerosol. Sonicate for 1 min, and dilute with *Diluent* to volume.

**Sample stock solution:** Fit the valve of the Topical Aerosol container with a cannula, and expel the contents of the container into a 100-mL, glass-stoppered, round-bottom flask. Attach the flask to a rotary evaporator, and evaporate under a vacuum of 600 mm of mercury for 2.5 h. Transfer a portion of the material in the round-bottom flask, equivalent to 1400 mg of benzocaine, to a 100-mL volumetric flask. Add 75 mL of methanol, and mix. Sonicate for 1 min, dilute with methanol to volume, and mix.

**Sample solution:** Transfer 10.0 mL of the *Sample stock solution* to a 100-mL volumetric flask. Add 75 mL of *Diluent*. Shake, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 313 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for benzocaine, butamben, and tetracaine are 0.3, 0.8, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2 between the benzocaine and butamben peaks; NLT 2 between the butamben and tetracaine peaks

**Relative standard deviation:** NMT 2.0% for each of the three analyte peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the individual percentage of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride

( $C_{15}H_{24}N_2O_2 \cdot HCl$ ) in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response of the corresponding analyte from the *Sample solution*

*r<sub>S</sub>* = peak response of the corresponding analyte from the *Standard solution*

*C<sub>S</sub>* = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of benzocaine, butamben, and tetracaine hydrochloride

### SPECIFIC TESTS

- **OTHER REQUIREMENTS:** It meets the requirements in *Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers* (601), *Pressure Test, Minimum Fill, and Leakage Test*.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in pressurized containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS (11)**  
USP Benzocaine RS  
USP Butamben RS  
USP Tetracaine Hydrochloride RS

## Benzocaine, Butamben, and Tetracaine Hydrochloride Gel

### DEFINITION

Benzocaine, Butamben, and Tetracaine Hydrochloride Gel is Benzocaine, Butamben, and Tetracaine Hydrochloride in a suitable gel base. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ).

### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Diluent:** Methanol and water (60:40)

**Mobile phase:** Methanol, water, and 0.25 M 1-heptanesulfonate (500:500:20)

**Standard solution:** Transfer 140 mg of USP Benzocaine RS to a 100-mL volumetric flask with the aid of 25 mL of methanol, and swirl. Transfer 140/*f* mg of USP Butamben RS to the same volumetric flask with the aid of 25 mL of water, *f* being the ratio of the labeled amount, as a percentage, of butamben to the labeled amount, as a percentage, of benzocaine in the Gel. Transfer 140/*f* mg of USP Tetracaine Hydrochloride RS, into the same volumetric flask with the aid of 25 mL of water, *f* being the ratio of the labeled amount, as a percentage, of tetracaine hydrochloride to the labeled amount, as a percentage, of benzocaine in the Gel. Sonicate for about 1 min, and dilute with *Diluent* to volume.

**Sample stock solution:** Nominally 14 mg/mL of benzocaine, prepared as follows. Transfer a portion of Gel, equivalent to 1400 mg of benzocaine, to a 100-mL volumetric flask. Add 75 mL of methanol, and mix. Soni-



cate for about 1 min, and dilute with methanol to volume.

**Sample solution:** Nominally 1.4 mg/mL of benzocaine in *Diluent* from the *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 313 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for benzocaine, butamben, and tetracaine are about 0.3, 0.8, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2 between benzocaine and butamben, and between butamben and tetracaine

**Relative standard deviation:** NMT 2.0% for each of the three analyte peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the corresponding analyte from the *Sample solution*

$r_S$  = peak response of the corresponding analyte from the *Standard solution*

$C_S$  = concentration of the corresponding Reference Standard in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.
- **USP REFERENCE STANDARDS (11)**
  - USP Benzocaine RS
  - USP Butamben RS
  - USP Tetracaine Hydrochloride RS

## Benzocaine, Butamben, and Tetracaine Hydrochloride Ointment

#### DEFINITION

Benzocaine, Butamben, and Tetracaine Hydrochloride Ointment is Benzocaine, Butamben, and Tetracaine Hydrochloride in a suitable ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ).

#### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Diluent:** Methanol and water (60:40)

**Mobile phase:** Methanol, water, and 0.25 M sodium 1-heptanesulfonate (500:500:20)

**Standard solution:** Transfer 140 mg of USP Benzocaine RS to a 100-mL volumetric flask with the aid of 25 mL of methanol, and swirl. Transfer 140 mg of USP Butamben RS to the same volumetric flask with the aid of 25 mL of water,  $J$  being the ratio of the labeled amount, as a percentage, of butamben to the labeled amount, as a percentage, of benzocaine in the Ointment. Transfer 140 mg of USP Tetracaine Hydrochloride RS to the same volumetric flask with the aid of 25 mL of water,  $J'$  being the ratio of the labeled amount, as a percentage, of tetracaine hydrochloride to the labeled amount, as a percentage, of benzocaine in the Ointment. Sonicate for about 1 min, and dilute with *Diluent* to volume.

**Sample stock solution:** Nominally 14 mg/mL of benzocaine, prepared as follows. Transfer a portion of Ointment, equivalent to 1400 mg of benzocaine, to a 100-mL volumetric flask. Add 75 mL of methanol, and mix. Sonicate for about 1 min, and dilute with methanol to volume.

**Sample solution:** Nominally 1.4 mg/mL of benzocaine in *Diluent* from the *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 313 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for benzocaine, butamben, and tetracaine are about 0.3, 0.8, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2 between benzocaine and butamben, and between butamben and tetracaine

**Relative standard deviation:** NMT 2.0% for each of the three analyte peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the corresponding analyte from the *Sample solution*

$r_S$  = peak response of the corresponding analyte from the *Standard solution*

$C_S$  = concentration of the corresponding Reference Standard in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.



- **USP REFERENCE STANDARDS (11)**
  - USP Benzocaine RS
  - USP Butamben RS
  - USP Tetracaine Hydrochloride RS

## Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution

### DEFINITION

Benzocaine, Butamben, and Tetracaine Hydrochloride Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ).

### IDENTIFICATION

- A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Diluent:** Methanol and water (60:40)

**Mobile phase:** Methanol, water, and 0.25 M sodium 1-heptanesulfonate (500:500:20)

**Standard solution:** Transfer 140 mg of USP Benzocaine RS to a 100-mL volumetric flask with the aid of 25 mL of methanol, and swirl. Transfer 140 mg of USP Butamben RS to the same volumetric flask with the aid of 25 mL of water,  $f$  being the ratio of the labeled amount, as a percentage, of butamben to the labeled amount, as a percentage, of benzocaine in the Topical Solution. Transfer 140 mg of USP Tetracaine Hydrochloride RS to the same volumetric flask with the aid of 25 mL of water,  $f'$  being the ratio of the labeled amount, as a percentage, of tetracaine hydrochloride to the labeled amount, as a percentage, of benzocaine in the Topical Solution. Sonicate for about 1 min, and dilute with *Diluent* to volume.

**Sample stock solution:** Nominally 14 mg/mL of benzocaine, prepared as follows. Transfer a portion of Topical Solution, equivalent to 1400 mg of benzocaine, to a 100-mL volumetric flask. Add 75 mL of methanol, and mix. Sonicate for about 1 min, and dilute with methanol to volume.

**Sample solution:** Nominally 1.4 mg/mL of benzocaine in *Diluent* from the *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 313 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for benzocaine, butamben, and tetracaine are about 0.3, 0.8, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2 between benzocaine and butamben, and between butamben and tetracaine

**Relative standard deviation:** NMT 2.0% for each of the three analyte peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of benzocaine ( $C_9H_{11}NO_2$ ), butamben ( $C_{11}H_{15}NO_2$ ), and tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of the corresponding analyte from the *Sample solution*
- $r_S$  = peak response of the corresponding analyte from the *Standard solution*
- $C_S$  = concentration of the corresponding Reference Standard in the *Standard solution* (mg/mL)
- $C_U$  = nominal concentration of the corresponding analyte in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.
- **USP REFERENCE STANDARDS (11)**
  - USP Benzocaine RS
  - USP Butamben RS
  - USP Tetracaine Hydrochloride RS

## Benzocaine and Menthol Topical Aerosol

### DEFINITION

Benzocaine and Menthol Topical Aerosol is a solution of Benzocaine and Menthol with suitable propellants in a pressurized container. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzocaine ( $C_9H_{11}NO_2$ ) and menthol ( $C_{10}H_{20}O$ ).

### IDENTIFICATION

- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The retention time of the major peak for benzocaine of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.** The retention time of the major peak for menthol of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • BENZOCAINE

**Solution A:** Dilute 1.0 mL of trifluoroacetic acid with water to 1 L.

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
15	72	28
18	50	50
18.1	90	10
20	90	10

**Diluent:** *Solution A* and *Solution B* (1:1)

**Standard solution:** 0.1 mg/mL of USP Benzocaine RS in *Diluent*. Sonicate for 2–5 min to dissolve before diluting to final volume.

**Sample solution:** Nominally 0.1 mg/mL of benzocaine in *Diluent* prepared as follows. Spray the contents of the Topical Aerosol into a flask with a stopper. Heat and stir the sprayed Topical Aerosol at 100° for 30 min in an oil bath to obtain a viscous liquid sample. Cool the sample to room temperature. Transfer an amount of Topical Aerosol equivalent to 10 mg of benzocaine to a 100-mL



volumetric flask. Dissolve the sample in *Diluent* and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm. For *Identification* test A, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 25-cm; 5-μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 μL

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzocaine ( $C_9H_{11}NO_2$ ) in the portion of Topical Aerosol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### • MENTHOL

Internal standard solution: 1 mg/mL of decanol in isopropyl alcohol

Standard stock solution: 1 mg/mL of USP Menthol RS in isopropyl alcohol

Standard solution: 0.5 mg/mL each of USP Menthol RS and decanol in isopropyl alcohol from *Internal standard solution* and *Standard stock solution*

Sample solution: Nominally 0.5 mg/mL of menthol prepared as follows. Spray the contents of the Topical Aerosol into a flask. Transfer an amount of Topical Aerosol equivalent to 5 mg of menthol to a 10-mL volumetric flask and add 5.0 mL of *Internal standard solution*. Dilute with isopropyl alcohol to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.53-mm × 30-m; fused-silica capillary column bonded with a 1.0-μm film of phase G16

Carrier gas: Hydrogen

#### Temperatures

Injection port: 250°

Detector: 250°

Column: See *Table 2*.

Table 2

Initial Temperature (°)	Hold Time at 130° (min)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
130	7.5	35	240	1.0

Flow rate: 10 mL/min

Injection volume: 1 μL

Injection type: Split, split ratio 10:1

#### System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for menthol and decanol are 1.0 and 1.6, respectively.]

#### Suitability requirements

Resolution: NLT 2.5 between the menthol and decanol peaks

Relative standard deviation: NMT 1.0% of the ratio of the peak response of menthol to that of decanol

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of menthol ( $C_{10}H_{20}O$ ) in the portion of Topical Aerosol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of menthol to decanol from the *Sample solution*

$R_S$  = peak response ratio of menthol to decanol from the *Standard solution*

$C_S$  = concentration of USP Menthol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of menthol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

• **MINIMUM FILL (755):** Meets the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

Solution A: Dilute 1.0 mL of trifluoroacetic acid with water to 1 L.

Solution B: Acetonitrile

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	90	10
34	50	50
35	90	10
38	90	10

Diluent: *Solution A* and *Solution B* (1:1)

Standard stock solution: 0.1 mg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent*. Sonicate for about 5 min to dissolve before diluting to final volume.

Standard solution: 0.001 mg/mL each of USP Benzocaine RS, USP Aminobenzoic Acid RS, and USP Ethyl 4-Nitrobenzoate RS in *Diluent* from the *Standard stock solution*

Sample solution: Nominally 0.5 mg/mL of benzocaine in *Diluent* prepared as follows. Spray the contents of the Topical Aerosol into a flask. Heat and stir the sprayed Topical Aerosol at 100° for 30 min in an oil bath to obtain a viscous liquid sample. Cool the sample to room temperature. Transfer an amount of Topical Aerosol equivalent to 50 mg of benzocaine to a 100-mL volumetric flask. Dissolve the sample in *Diluent* and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Flow rate: 1.5 mL/min

Injection volume: 20 μL

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Relative standard deviation: NMT 2.0% for each peak corresponding to benzocaine, aminobenzoic acid, and ethyl 4-nitrobenzoate



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of aminobenzoic acid and ethyl 4-nitrobenzoate in the portion of Topical Aerosol taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of aminobenzoic acid or ethyl 4-nitrobenzoate from the *Sample solution*  
 $r_s$  = peak response of the corresponding Reference Standard from the *Standard solution*  
 $C_s$  = concentration of USP Aminobenzoic Acid RS or USP Ethyl 4-Nitrobenzoate RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Topical Aerosol taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of each unspecified degradation product from the *Sample solution*  
 $r_s$  = peak response of benzocaine from the *Standard solution*  
 $C_s$  = concentration of USP Benzocaine RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of benzocaine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 4. Disregard peaks less than 0.05%.

**Table 4**

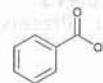
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Aminobenzoic acid	0.3	0.2
Benzocaine	1.0	—
Ethyl 4-nitrobenzoate	2.1	0.2
Any other unspecified degradation product	—	0.1
Total degradation products	—	2.0

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **PRESSURE TEST:** It meets the requirements in *Topical Aerosols* (603).
- **LEAKAGE TEST:** It meets the requirements in *Leak Rate* (604).

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, pressurized containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS** (11)
  - USP Aminobenzoic Acid RS
  - Benzoic acid, 4-amino.
  - $C_7H_7NO_2$  137.14
  - USP Benzocaine RS
  - USP Ethyl 4-Nitrobenzoate RS
  - Benzoic acid, 4-nitro-, ethyl ester.
  - $C_9H_9NO_4$  195.17

**USP Menthol RS****Benzoic Acid** $C_7H_6O_2$ 

122.12

Benzoic acid [65-85-0].

**DEFINITION**

Benzoic Acid contains NLT 99.5% and NMT 100.5% of benzoic acid ( $C_7H_6O_2$ ), calculated on the anhydrous basis.

**IDENTIFICATION**• **A.**

**Sample solution:** Prepare a saturated solution of Benzoic Acid in water, and filter twice.

**Analysis 1:** To one portion of the filtrate add ferric chloride TS.

**Acceptance criteria 1:** A salmon-colored precipitate is formed.

**Analysis 2:** To a separate 10-mL portion of the filtrate add 1 mL of 7 N sulfuric acid, and cool the mixture.

**Acceptance criteria 2:** A white precipitate forms in 10 min. This precipitate is soluble in ether.

**ASSAY**• **PROCEDURE**

**Sample:** 500 mg of Benzoic Acid

**Analysis:** Dissolve the *Sample* in 25 mL of diluted alcohol that previously has been neutralized with 0.1 N sodium hydroxide. Add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS to a pink color. Each mL of 0.1 N sodium hydroxide is equivalent to 12.21 mg of benzoic acid ( $C_7H_6O_2$ ).

**Acceptance criteria:** 99.5%–100.5% on the anhydrous basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.05%

**Delete the following:**• **HEAVY METALS** (231)

**Test preparation:** Dissolve 2.0 g in 25 mL of acetone, and add 2 mL of water.

**Analysis:** Add 1.2 mL of thioacetamide–glycerin base TS and 2 mL of pH 3.5 Acetate Buffer, and allow to stand for 5 min.

**Acceptance criteria:** NMT 10 µg/g; any color produced is not darker than that of a control made with 25 mL of acetone and 2.0 mL of *Standard Lead Solution* and treated in the same manner. • (Official 1-Jan-2018)

**SPECIFIC TESTS**

- **CONGEALING TEMPERATURE** (651): 121°–123°

- **WATER DETERMINATION, Method I** (921)

**Sample solution:** A 1-in-2 solution of methanol in pyridine is used as the solvent.

**Acceptance criteria:** NMT 0.7%

- **READILY CARBONIZABLE SUBSTANCES TEST** (271)

**Sample solution:** 500 mg in 5 mL of sulfuric acid

**Acceptance criteria:** The solution has no more color than *Matching Fluid Q*.

- **READILY OXIDIZABLE SUBSTANCES**

**Sample solution:** Add 1.5 mL of sulfuric acid to 100 mL of water. Heat to boiling, and add 0.1 N potassium permanganate, dropwise, until the pink color persists



for 30 s. Dissolve 1.00 g of Benzoic Acid in the hot solution.

**Analysis:** Titrate with 0.1 N potassium permanganate VS to a pink color that persists for 15 s.

**Acceptance criteria:** NMT 0.50 mL of 0.10 N potassium permanganate is consumed.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## Benzoic and Salicylic Acids Ointment

### DEFINITION

Benzoic and Salicylic Acids Ointment is Benzoic Acid and Salicylic Acid, present in a ratio of 2:1, in a suitable ointment base. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of benzoic acid ( $C_7H_6O_2$ ) and salicylic acid ( $C_7H_6O_3$ ).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHY

**Diluent:** Mixture of chloroform and methanol (1:1)

**Standard solution A:** 2.4 mg/mL of USP Benzoic Acid RS in *Diluent*

**Standard solution B:** 1.2 mg/mL of USP Salicylic Acid RS in *Diluent*

**Sample solution:** Equivalent to 60 mg of benzoic acid and 30 mg of salicylic acid from Ointment, in 25 mL of *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu$ L of each solution at separate points 2.5 cm from the bottom edge of a 20-  $\times$  20-cm thin-layer chromatographic plate

**Developing solvent system:** Chloroform, acetone, isopropyl alcohol, methanol, and ammonium hydroxide (30:30:15:15:10)

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, mark the solvent front, and allow the solvent to evaporate. View the chromatogram under short-wavelength (254 nm) UV radiation.

**Acceptance criteria:** The two major fluorescent spots from the *Sample solution* correspond in color and in  $R_f$  value to those from *Standard solution A* and *Standard solution B*.

### ASSAY

#### • PROCEDURE

**Ferric chloride-urea reagent:** On the day of use, dissolve, without heating, 18 g of urea in a mixture of 2.5 mL of ferric chloride solution (6 in 10) and 12.5 mL of 0.05 N hydrochloric acid.

**Column A:** Insert a small pledget of glass wool above the stem constriction of a 20-  $\times$  2.5-cm chromatographic tube. Mix 1 g of chromatographic siliceous earth with 0.5 mL of dilute phosphoric acid (3 in 10) to form a uniform, fluffy mixture; transfer to the chromatographic tube; and pack evenly over the glass wool, exerting gentle pressure. Similarly, mix 4 g of chromatographic siliceous earth with 3 mL of *Ferric chloride-urea*

*reagent*, and pack uniformly over the first layer. Cover the column with a pad of glass wool.

**Column B:** Insert a small pledget of glass wool above the stem constriction of a second 20-  $\times$  2.5-cm chromatographic tube. Mix 4 g of chromatographic siliceous earth with 2 mL of sodium bicarbonate solution (1 in 12), prepared just before use, to a uniform, fluffy mixture; and pack evenly over the glass wool. Cover the column with a pad of glass wool.

**Diluent:** Glacial acetic acid in chloroform (3 in 100)

**Standard solution A:** 20  $\mu$ g/mL of USP Salicylic Acid RS in *Diluent*

**Standard solution B:** 40  $\mu$ g/mL of USP Benzoic Acid RS in *Diluent*

**Sample solution:** Transfer an amount of the Ointment, equivalent to 100 mg of benzoic acid and 50 mg of salicylic acid, to a 250-mL volumetric flask, and dissolve in 150 mL of chloroform by warming on a steam bath. Cool. Dilute with chloroform to volume to obtain a solution having a nominal concentration of 200  $\mu$ g/mL of salicylic acid and 400  $\mu$ g/mL of benzoic acid.

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Mount *Column A* directly over *Column B*, then pipet 10 mL of *Sample solution* onto *Column A*, and allow it to pass into the column. Wash the columns with two 40-mL portions of chloroform, allowing the first portion to recede to the top of each column before adding the second portion. Discard the eluates, and separate the columns.

**Salicylic acid content:** Elute *Column A* with 95 mL of *Diluent*, collecting the eluate in a 100-mL volumetric flask. Dilute the contents of the flask with *Diluent* to volume, and mix. Concomitantly determine the absorbances of the eluate and *Standard solution A* in 1-cm cells at the wavelength of maximum absorbance at 311 nm, with a suitable spectrophotometer, using *Diluent* as the blank.

Calculate the percentage of the labeled amount of salicylic acid ( $C_7H_6O_3$ ) in the portion of Ointment taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times F \times 100$$

$A_U$  = absorbance of the diluted eluate from *Column A*

$A_S$  = absorbance of *Standard solution A*

$C_S$  = concentration of USP Salicylic Acid RS in *Standard solution A* ( $\mu$ g/mL)

$C_U$  = nominal concentration of the salicylic acid in the *Sample solution* ( $\mu$ g/mL)

$F$  = sample dilution factor, 10

**Acceptance criteria:** 90.0%–110.0%

**Benzoic acid content:** Elute *Column B* with 95 mL of *Diluent*, collecting the eluate in a 100-mL volumetric flask. Dilute the contents of the flask with *Diluent* to volume, and mix. Concomitantly determine the absorbances of eluate and *Standard solution B* in 1-cm cells at the wavelength of maximum absorbance at 275 nm, with a suitable spectrophotometer, using *Diluent* as the blank.

Calculate the percentage of the labeled amount of benzoic acid ( $C_7H_6O_2$ ) in the portion of Ointment taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times F \times 100$$

$A_U$  = absorbance of the diluted eluate from *Column B*

$A_S$  = absorbance of *Standard solution B*

$C_S$  = concentration of USP Benzoic Acid RS in *Standard solution B* ( $\mu$ g/mL)

$C_U$  = nominal concentration of benzoic acid in the *Sample solution* ( $\mu$ g/mL)

$F$  = sample dilution factor, 10



Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and avoid exposure to temperatures exceeding 30°.
- **LABELING:** Label Ointment to indicate the concentrations of Benzoic Acid and Salicylic Acid and to indicate whether the ointment base is water-soluble or water-insoluble.
- **USP REFERENCE STANDARDS (11)**  
USP Benzoic Acid RS  
USP Salicylic Acid RS

# Benzoin

## DEFINITION

Benzoin is the balsamic resin obtained from *Styrax benzoin* Dryand. or *Styrax paralleloneurus* Perkins, known in commerce as Sumatra Benzoin, or from *Styrax tonkinensis* (Pierre) Craib ex Hartwich, or other species of the Section *Anthostyrax* of the genus *Styrax*, known in commerce as Siam Benzoin (Fam. *Styracaceae*).

Sumatra Benzoin yields NLT 75.0% of alcohol-soluble extractive, and Siam Benzoin yields NLT 90.0% of alcohol-soluble extractive.

## IDENTIFICATION

- **A.** A solution in alcohol becomes milky upon the addition of water, and the mixture is acid to litmus paper.
- **B. IDENTIFICATION OF ARTICLES OF BOTANICAL ORIGIN (563)**  
**Analysis:** Heat a few fragments in a test tube.  
**Acceptance criteria:** Sumatra Benzoin evolves a sublimate consisting of plates and small, rod-like crystals of cinnamic acid and its esters that strongly polarize light. Siam Benzoin evolves a sublimate directly above the melted mass, consisting of numerous long, rod-shaped crystals of benzoic acid that do not strongly polarize light.

## ASSAY

### PROCEDURE

**Sample:** Place 2 g of Benzoin in a tared extraction thimble, and insert the thimble in a continuous-extraction apparatus. Place 100 mg of sodium hydroxide in the receiving flask of the apparatus, and extract the Benzoin with alcohol for 5 h, or until completely extracted. Dry the extraction thimble containing the insoluble residue at 105° for 2 h.

**Analysis:** On a separate portion of Benzoin, determine the water content as directed for *Water Determination (921), Method II*. Calculate the weight of water in the quantity of the Benzoin taken for the Assay, and subtract it from the original weight of the Benzoin taken. The difference between this result and the weight of the residue in the extraction thimble represents the alcohol-soluble extractive.

**Acceptance criteria:** The alcohol-soluble extractive is NLT 75.0% for Sumatra Benzoin and NLT 90.0% for Siam Benzoin.

## OTHER COMPONENTS

### CONTENT OF BENZOIC ACID

**Analysis:** Treat 1 g of powdered Benzoin with 15 mL of warm carbon disulfide. Filter through a small pledget of cotton, wash the cotton with an additional 5 mL of carbon disulfide, and allow the filtrate to evaporate spontaneously.

**Acceptance criteria:** The weight of the residue is NLT 6.0% of the weight of Benzoin taken for Sumatra Benzoin and NLT 12.0% for Siam Benzoin. This residue meets the requirements for *Identification Tests—General (191), Benzoate*.

## IMPURITIES

### Inorganic Impurities

- **ARTICLES OF BOTANICAL ORIGIN, Acid-Insoluble Ash (561):** NMT 1.0% in Sumatra Benzoin; NMT 0.5% in Siam Benzoin

### Organic Impurities

- **PROCEDURE: ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter (561):** NMT 1.0% in Siam Benzoin

## SPECIFIC TESTS

### BOTANIC CHARACTERISTICS

**Sumatra Benzoin:** Blocks or lumps of varying size, made up of tears, compacted together, with a reddish brown, reddish gray, or grayish brown resinous mass; the tears are externally yellowish or rusty brown, milky white on fresh fracture; hard and brittle at ordinary temperatures, but softened by heat.

**Siam Benzoin:** Pebble-like tears of variable size and shape, compressed, yellowish brown to rusty brown externally, milky white on fracture, separate or very slightly agglutinated; hard and brittle at ordinary temperatures, but softened by heat.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate whether it is Sumatra Benzoin or Siam Benzoin.

# Compound Benzoin Tincture

## DEFINITION

Prepare Compound Benzoin Tincture as follows.

Benzoin, in moderately coarse powder	100 g
Aloe, in moderately coarse powder	20 g
Storax	80 g
Tolu Balsam	40 g
Alcohol, a sufficient quantity to make	1000 mL

Macerate the ingredients with 750 mL of *Alcohol* in a container that can be closed, and put in a warm place. Agitate it frequently during 3 days or until the soluble matter is dissolved. Transfer the mixture to a filter. When most of the liquid has drained away, wash the residue on the filter with a sufficient quantity of *Alcohol*, combining the filtrates to produce 1000 mL of Tincture, and mix.

## OTHER COMPONENTS

- **ALCOHOL DETERMINATION (611), Method II:** 74.0%–80.0% of alcohol ( $C_2H_5OH$ ), the dilution made with methanol instead of with water, to approximately 2% alcohol

## SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 0.870–0.885

### LIMIT OF NONVOLATILE RESIDUE

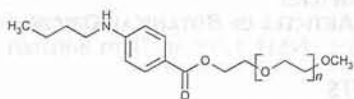
**Analysis:** Evaporate 3 mL of Tincture in a suitable tared dish on a steam bath, and dry the residue at 100° for 2 h.

**Acceptance criteria:** The weight of the residue is 525–675 mg.



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.
- **LABELING:** Label it to indicate that it is flammable.

**Benzonate**

$C_{30}H_{53}NO_{11}$  (av.) 603.74 (average)  
Benzoic acid, 4-(butylamino)-, 2,5,8,11,14,17,20,23,26-nonaooctacos-28-yl ester.  
2,5,8,11,14,17,20,23,26-Nonaooctacosan-28-yl-p-(butylamino)benzoate [104-31-4].

» Benzonate contains not less than 95.0 percent and not more than 105.0 percent of  $C_{30}H_{53}NO_{11}$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Benzonate RS

**Identification**—

A: Infrared Absorption (197F).

B: Ultraviolet Absorption (197U)—

Solution: 15 µg per mL.

Medium: water.

**Refractive index** (831): between 1.509 and 1.511 at 20°.

**Water Determination, Method I** (921): not more than 0.3%.

**Residue on ignition** (281): not more than 0.1%.

**Chloride** (221)—Mix 20 mL of a solution (1 in 10) with 20 mL of water and 1 mL of nitric acid, shake for 1 hour, and allow to stand for 1 hour. Pass through a filter having a porosity of 0.2 µm, and to the filtrate add 1 mL of silver nitrate TS. Dilute with water to 50 mL, mix, and allow to stand protected from light for 10 minutes: the turbidity does not exceed that produced by 0.10 mL of 0.020 N hydrochloric acid (0.0035%).

**Sulfate** (221)—Mix 5 mL of a solution (1 in 20) with 5 mL of water and 1 mL of 3 N hydrochloric acid, shake for 1 hour, and allow to stand for 1 hour. Pass through a filter having a porosity of 0.2 µm, and to the filtrate add 1 mL of barium chloride TS. Mix, and allow to stand for 10 minutes: the turbidity does not exceed that produced by 0.10 mL of 0.020 N sulfuric acid (0.04%).

**Delete the following:**

• **Heavy metals, Method II** (231): 0.001%. (Official 1-Jan-2018)

**Assay**—Weigh accurately about 5 g of Benzonate, and reflux with 25.0 mL of 0.5 N sodium hydroxide VS for 1 hour. Cool, add 25 mL of water and 10 drops of bromothymol blue TS, and titrate the excess alkali with 0.5 N hydrochloric acid VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.5 N sodium hydroxide is equivalent to 301.5 mg of  $C_{30}H_{53}NO_{11}$ .

**Benzonate Capsules**

» Benzonate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of benzonate ( $C_{30}H_{53}NO_{11}$  av.).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Benzonate RS

**Identification**—

A: The contents of the Capsules meet the requirements of *Identification* test A under *Benzonate*. If a difference is observed, or if excipients are present, use an amount of the contents of Capsules equivalent to about 100 mg of benzonate, mixed with 25 mL of 0.01 N hydrochloric acid, and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with "Transfer the liquid to a separator."

B: The contents of the Capsules respond to *Identification* test B under *Benzonate*.

**Dissolution** (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

Determine the amount of benzonate dissolved by employing the following method.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and 0.04 M monobasic potassium phosphate (3:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Transfer 50 mg, accurately weighed, of USP Benzonate RS to a 100-mL volumetric flask, and add about 50 mL of water. Sonicate for 10 minutes, cool, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, and dilute with water to volume.

**Test solution**—Pass a portion of the solution under test through a 0.45-µm filter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 310-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 15 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of benzonate dissolved by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times LC}$$

in which  $r_U$  and  $r_S$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of USP Benzonate RS in the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; and  $LC$  is the Tablet label claim, in mg.

**Tolerances**—Not less than 80% (Q) of the labeled amount of benzonate is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.



**Assay—**

**Standard preparation**—Transfer about 50 mg of USP Benzonate RS, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix.

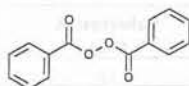
**Assay preparation**—Mix a number of Capsules, equivalent to about 500 mg of benzonate, with 40 mL of chloroform in a suitable high-speed blender, and dilute with chloroform to 100.0 mL. Transfer 10.0 mL of this solution, equivalent to about 50 mg of benzonate, to a 100-mL volumetric flask, and evaporate the chloroform on a steam bath with the aid of a current of air. Dissolve the residue in water, dilute with water to volume, and mix.

**Procedure**—Transfer 4.0 mL each of the *Standard preparation*, the *Assay preparation*, and water to provide the blank, to separate test tubes. To each tube add in succession 1.0 mL of 1 M hydroxylamine hydrochloride and 1.0 mL of 3.5 N sodium hydroxide, mixing after each addition. Allow to stand for 10 minutes, accurately timed, then add 1.0 mL of 3.5 N hydrochloric acid, mix, add 1.0 mL of ferric chloride solution (8 in 100), and mix. Allow to stand for 30 minutes, accurately timed. Gently swirl the tubes for 1 minute to remove any gas bubbles present, then concomitantly determine the absorbances of the solutions in 1-cm cells, at the wavelength of maximum absorbance at about 500 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the quantity, in mg, of benzonate ( $C_{30}H_{53}NO_{11}$  av.) in the number of Capsules taken by the formula:

$$C(A_u / A_s)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Benzonate RS in the *Standard preparation*; and  $A_u$  and  $A_s$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Hydrous Benzoyl Peroxide



$C_{14}H_{10}O_4$  (anhydrous)  
Peroxide, dibenzoyl;  
Benzoyl peroxide [94-36-0].

242.23

**DEFINITION**

Hydrous Benzoyl Peroxide contains NLT 90.0% and NMT 110.0% of the labeled amount of  $C_{14}H_{10}O_4$ . It contains a minimum of 20% of water for the purpose of reducing flammability and shock sensitivity.

**[CAUTION]**—Hydrous Benzoyl Peroxide may explode at temperatures higher than 60° or cause fires in the presence of reducing substances. Store it in the original container, treated to reduce static charges.]

**IDENTIFICATION**

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 10 mg/mL of Hydrous Benzoyl Peroxide, previously subjected to the Assay, in methanol

**Sample solution:** 10 mg/mL of benzoyl peroxide in methanol

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu\text{L}$

**Developing solvent system:** Toluene, dichloromethane, and glacial acetic acid (50:2:1)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Place the plate in a developing chamber containing and equilibrated with the *Developing solvent system*. Develop the chromatogram until the solvent front has moved three-fourths of the length of the plate. Remove the plate, and allow the solvent to evaporate. Observe the plate under short-wavelength UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

- **B.** The *Sample solution* in the test for *Organic Impurities* exhibits a major peak for benzoyl peroxide, the retention time of which corresponds to that exhibited by the *Standard solution*.

**ASSAY**

• **PROCEDURE**

**Sample:** 300 mg of previously mixed Hydrous Benzoyl Peroxide in a conical flask fitted with a ground-glass stopper. Weigh again to obtain the weight of the *Sample*.

**Analysis:** Add 30 mL of glacial acetic acid, previously sparged with carbon dioxide for NLT 2 min just before use, and swirl the flask gently to dissolve. Add 5 mL of potassium iodide solution (1 in 2), and mix. Allow the solution to stand for 1 min. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS. As the endpoint is approached, add 1 drop of starch iodide paste TS, or equivalent, and continue the titration to the discharge of the blue color. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N sodium thiosulfate is equivalent to 12.11 mg of  $C_{14}H_{10}O_4$ .

**Acceptance criteria:** 90.0%–110.0% of the labeled amount

**IMPURITIES****Organic Impurities**

• **PROCEDURE**

**Solution A:** Prepare a mixture of acetonitrile and glacial acetic acid (1000:1).

**Solution B:** Prepare a mixture of water and glacial acetic acid (1000:1).

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	18	82
20	60	40
30	60	40

**System suitability solution:** 100  $\mu\text{g}$ /mL of benzoic acid and 60  $\mu\text{g}$ /mL of methylparaben in acetonitrile

**Standard solution:** Dissolve a quantity of Hydrous Benzoyl Peroxide, previously subjected to the Assay, in acetonitrile to obtain a solution containing 0.32 mg/mL.

**Sample solution:** 0.32 mg/mL of benzoyl peroxide in acetonitrile

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection size:** 10  $\mu\text{L}$

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between benzoic acid and methylparaben

**Tailing factors:** NMT 2.0 for the benzoic acid and methylparaben peaks



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the area, as a percentage, of each peak in the chromatogram of the *Sample solution*:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for any individual peak other than the principal peak in the *Sample solution*

$r_T$  = sum of the peak responses of all the individual peaks including the principal peak in the *Sample solution*

**Acceptance criteria:** The area of any individual peak other than the principal peak is NMT 1.5% of the total area. The sum of the areas of all peaks other than the principal peak is NMT 2.0% of the total area.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Store in the original container, at room temperature. [NOTE—Do not transfer Hydrous Benzoyl Peroxide to metal or glass containers fitted with friction tops. Do not return unused material to its original container, but destroy it by treatment with sodium hydroxide solution (1 in 10) until addition of a crystal of potassium iodide results in no release of free iodine.]

**Benzoyl Peroxide Gel****DEFINITION**

Benzoyl Peroxide Gel is benzoyl peroxide in a suitable gel base. It contains NLT 90.0% and NMT 125.0% of the labeled amount of benzoyl peroxide ( $C_{14}H_{10}O_4$ ).

**IDENTIFICATION**

**A.** The retention time of the major peak for benzoyl peroxide of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Acetonitrile in water (5 in 10)

**Internal standard solution:** 3.6 mg/mL of ethyl benzoate in acetonitrile

**Standard stock solution:** 0.8 mg/mL of benzoyl peroxide prepared as follows. Transfer a suitable quantity of benzoyl peroxide, recently subjected to the Assay in Hydrous Benzoyl Peroxide, into a weighed conical flask fitted with a glass stopper. Weigh again to obtain the weight of the specimen, and quantitatively dissolve in acetonitrile.

**Standard solution:** 0.32 mg/mL of benzoyl peroxide prepared as follows. Mix 10 mL of *Standard stock solution* and 5 mL of *Internal standard solution*, and dilute with acetonitrile to 25 mL.

**Sample stock solution:** Transfer an equivalent to 40 mg of benzoyl peroxide from Gel into a 50-mL volumetric flask, add 40 mL of acetonitrile, and shake until the material is thoroughly dispersed. Sonicate the mixture for 5 min, dilute with acetonitrile to volume, mix, and filter.

**Sample solution:** 10 mL of *Sample stock solution* and 5 mL of *Internal standard solution*; dilute with acetonitrile to 25 mL.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

**System suitability**

**Sample:** *Standard solution* (three replicate injections)

[NOTE—The retention times for ethyl benzoate and benzoyl peroxide are 7 and 14 min, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between ethyl benzoate and benzoyl peroxide

**Tailing factors:** NMT 2.0 for the ethyl benzoate and benzoyl peroxide peaks

**Peak response ratios:** The lowest and highest peak response ratios ( $R_S$ ) agree within 2.0%.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzoyl peroxide ( $C_{14}H_{10}O_4$ ) in the portion of Gel taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of benzoyl peroxide to ethyl benzoate from the *Sample solution*

$R_S$  = peak response ratio of benzoyl peroxide to ethyl benzoate from the *Standard solution*

$C_S$  = concentration of benzoyl peroxide in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzoyl peroxide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–125.0%

**IMPURITIES**• **ORGANIC IMPURITIES**

**Solution A:** Acetonitrile and glacial acetic acid (1000:1)

**Solution B:** Water and glacial acetic acid (1000:1)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	18	82
20	60	40
30	60	40

**System suitability solution:** 100 µg/mL of benzoic acid and 60 µg/mL of methylparaben in acetonitrile

**Standard solution A:** 500 µg/mL of benzoic acid in acetonitrile

**Standard solution B:** 20 µg/mL of ethyl benzoate in acetonitrile

**Standard solution C:** 20 µg/mL of benzaldehyde in acetonitrile

**Standard solution D:** Equivalent to 40 µg/mL of anhydrous benzoyl peroxide in acetonitrile, prepared from hydrous benzoyl peroxide, which has been analyzed as follows. Place 300 mg of previously mixed hydrous benzoyl peroxide in a conical flask fitted with a ground-glass stopper. Weigh again to obtain the weight of the sample. Add 30 mL of glacial acetic acid, previously sparged with carbon dioxide for NLT 2 min just before use, and swirl the flask gently to dissolve. Add 5 mL of potassium iodide solution (1 in 2), and mix. Allow the solution to stand for 1 min. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS. As the endpoint is approached, add 1 drop of starch iodide paste TS, or equivalent, and continue the titration to the discharge of the blue color. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N sodium thiosulfate is equivalent to 12.11 mg of anhydrous benzoyl peroxide ( $C_{14}H_{10}O_4$ ).



**Sample solution:** Transfer an amount of Gel equivalent to 100 mg of benzoyl peroxide to a 50-mL volumetric flask, and add 25 mL of acetonitrile. Shake vigorously to disperse the specimen, sonicate for 5 min, dilute with acetonitrile to volume, and filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between benzoic acid and methylparaben

**Tailing factor:** NMT 2.0 for the benzoic acid and methylparaben peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** The responses of any peaks from the *Sample solution* corresponding to benzoic acid, ethyl benzoate, and benzaldehyde are NMT those of the main peaks from *Standard solution A* (25%), *Standard solution B* (1%), and *Standard solution C* (1%), respectively. The response of any other impurity peak from the *Sample solution*—other than the main benzoyl peroxide peak, any benzoic acid, ethyl benzoate, benzaldehyde, methylparaben, or propylparaben peak, and any solvent peak—is NMT that from *Standard solution D* (2%); and the sum of the responses of all the impurity peaks—other than those of benzoic acid, ethyl benzoate, and benzaldehyde—is NMT that from *Standard solution D* (2%).

#### SPECIFIC TESTS

• **PH** (791): 2.8–6.6

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

## Benzoyl Peroxide Lotion

#### DEFINITION

Benzoyl Peroxide Lotion is benzoyl peroxide in a suitable lotion base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of benzoyl peroxide ( $C_{14}H_{10}O_4$ ).

#### IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

**Mobile phase:** Acetonitrile in water (5 in 10)

**Internal standard solution:** 3.6 mg/mL of ethyl benzoate in acetonitrile

**Standard stock solution:** Transfer a suitable quantity of benzoyl peroxide, recently subjected to the Assay under *Hydrous Benzoyl Peroxide*, in a weighed conical flask fitted with a glass stopper. Weigh again to obtain the weight of the specimen, and quantitatively dissolve in acetonitrile to obtain a solution containing 0.8 mg/mL.

**Standard solution:** 10 mL of *Standard stock solution* and 5 mL of *Internal standard solution*. Dilute with acetonitrile to 25 mL. This *Standard solution* contains 0.32 mg/mL of benzoyl peroxide.

**Sample stock solution:** Transfer the equivalent to 40 mg of benzoyl peroxide from Lotion in a 50-mL volumetric flask, and add 40 mL of acetonitrile. Shake vigorously until the material is thoroughly dispersed. Sonicate the mixture for 5 min, dilute with acetonitrile to volume, mix, and filter.

ously until the material is thoroughly dispersed. Sonicate the mixture for 5 min, dilute with acetonitrile to volume, mix, and filter.

**Sample solution:** 10 mL of *Sample stock solution* and 5 mL of *Internal standard solution*. Dilute with acetonitrile to 25 mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution* (three replicate injections)

[NOTE—The retention times for ethyl benzoate and benzoyl peroxide are 7 and 14 min, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between ethyl benzoate and benzoyl peroxide

**Tailing factor:** NMT 2.0 for the ethyl benzoate and benzoyl peroxide peaks

**Peak response ratios:** The lowest and highest peak response ratios ( $R_s$ ) agree within 2.0%.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzoyl peroxide ( $C_{14}H_{10}O_4$ ) in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of benzoyl peroxide to ethyl benzoate from the *Sample solution*

$R_S$  = peak response ratio of benzoyl peroxide to ethyl benzoate from the *Standard solution*

$C_S$  = concentration of benzoyl peroxide in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of benzoyl peroxide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### IMPURITIES

##### ORGANIC IMPURITIES

**Solution A:** Acetonitrile and glacial acetic acid (1000:1)

**Solution B:** Glacial acetic acid and water (1:1000)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	18	82
20	60	40
30	60	40

**System suitability solution:** 100 µg/mL of benzoic acid and 60 µg/mL of methylparaben in acetonitrile

**Standard solution A:** 500 µg/mL of benzoic acid in acetonitrile

**Standard solution B:** 20 µg/mL of ethyl benzoate in acetonitrile

**Standard solution C:** 20 µg/mL of benzaldehyde in acetonitrile

**Standard solution D:** Prepare a solution of hydrous benzoyl peroxide, previously subjected to the Assay under *Hydrous Benzoyl Peroxide*, in acetonitrile containing the equivalent of 40 µg/mL of anhydrous benzoyl peroxide.

**Sample solution:** Equivalent to 100 mg of benzoyl peroxide from Lotion. In a 50-mL volumetric flask add 25 mL of acetonitrile, and shake vigorously to disperse the specimen. Sonicate for 5 min, dilute with acetonitrile to volume, mix, and filter.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.2 mL/min

Injection volume: 10 µL

**System suitability**Sample: *System suitability solution***Suitability requirements**

Resolution: NLT 2.0 between benzoic acid and methylparaben

Tailing factor: NMT 2.0 for the benzoic acid and methylparaben peaks

**Analysis**Samples: *Standard solution* and *Sample solution*

**Acceptance criteria:** The responses of any peaks from the *Sample solution* corresponding to benzoic acid, ethyl benzoate, and benzaldehyde are NMT those of the main peaks from *Standard solution A* (25%), *Standard solution B* (1%), and *Standard solution C* (1%), respectively. The response of any other impurity peak from the *Sample solution*, other than the main benzoyl peroxide peak, any benzoic acid, ethyl benzoate, benzaldehyde, methylparaben, or propylparaben peak, and any solvent peak, is NMT that from *Standard solution D* (2%); and the sum of the responses of all the impurity peaks, other than those of benzoic acid, ethyl benzoate, and benzaldehyde, is NMT that from *Standard solution D* (2%).

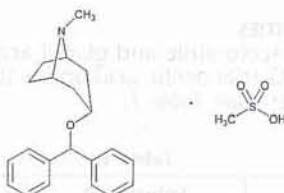
**SPECIFIC TESTS**

- **PH** (791): 2.8–6.6

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

## Benztropine Mesylate



$C_{21}H_{25}NO \cdot CH_4O_3S$  403.53  
 8-Azabicyclo[3.2.1]octane, 3-(diphenylmethoxy)-N-methyl-, endo-, methanesulfonate;  
 3α-(Diphenylmethoxy)-1αH,5αH-tropane methanesulfonate [132-17-2].

**DEFINITION**

Benztropine Mesylate contains NLT 98.0% and NMT 100.5% of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)

**ASSAY**

- **PROCEDURE**

Sample: 60 mg of Benztropine Mesylate

**Analysis:** Dissolve the *Sample* in 25 mL of water, add 5 mL of sodium carbonate TS, and extract with four 10-mL portions of chloroform. Wash the combined chloroform extracts with about 10 mL of water, and extract the wash solution with 5 mL of chloroform. Filter the combined chloroform extracts through a tightly

packed pledget of cotton, and wash the cotton with about 5 mL of chloroform. Add methyl red TS, and titrate the chloroform solution with 0.01 N perchloric acid in dioxane VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.01 N perchloric acid is equivalent to 4.035 mg of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ).  
**Acceptance criteria:** 98.0%–100.5% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE** (741): 141°–148°

- **LOSS ON DRYING** (731)

Analysis: Dry a sample at 105° for 2 h.

Acceptance criteria: NMT 5.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Benztropine Mesylate RS

## Benzotropine Mesylate Injection

**DEFINITION**

Benzotropine Mesylate Injection is a sterile solution of Benztropine Mesylate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ).

**IDENTIFICATION**

- **A.**

**Standard stock solution:** 0.2 mg/mL of USP Benztropine Mesylate RS

**Standard solution:** In a separator containing the *Standard stock solution* add 2 mL of 1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

**Sample stock solution:** Dilute a volume of Injection, equivalent to 10 mg of benztropine mesylate, in a separator to 50 mL with water (0.2 mg/mL).

**Sample solution:** In a separator containing the *Sample stock solution* add 2 mL of 1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

**Chromatographic system**

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 1 µL

**Developing solvent system:** Chloroform, methanol, and a 1-in-4 solution of ammonium hydroxide (40:10:1)

**Analysis**Samples: *Standard solution* and *Sample solution*

Allow the applications to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with potassium iodoplatinate TS.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.



**ASSAY****• PROCEDURE**

**Buffer:** Transfer 0.83 mL of octylamine to a 1-L volumetric flask, dilute with water to volume, and adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Acetonitrile and *Buffer* (65:35)

**Standard solution:** 1 mg/mL of USP Bzotroprine Mesylate RS

**Sample solution:** Nominally 1 mg/mL of bztroprine mesylate from the volume of Injection

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 259 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1.3 mL/min adjusted, as needed, to obtain a retention time of 7 min for bztroprine mesylate

**Injection volume:** 25 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of labeled amount of bztroprine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ) in each mL of the Injection:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bzotroprine Mesylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bztroprine mesylate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

• **BACTERIAL ENDOTOXINS TEST** (85): NMT 55.6 USP Endotoxin Units/mg of bztroprine mesylate

• **pH** (791): 5.0–8.0

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** (11)

USP Bzotroprine Mesylate RS

USP Endotoxin RS

mesylate, in 50 mL of water, shake by mechanical means for 30 min, and filter into a separator (0.2 mg/mL).

**Sample solution:** In a separator containing the *Sample stock solution* add 2 mL of 1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

**Chromatographic system**

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 1 µL

**Developing solvent system:** Chloroform, methanol, and a 1-in-4 solution of ammonium hydroxide (40:10:1)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Allow the applications to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with potassium iodoplatinate TS.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

**ASSAY****• PROCEDURE**

**Buffer:** Transfer 0.83 mL of octylamine to a 1-L volumetric flask, dilute with water to volume, and adjust with phosphoric acid to a pH of 3.0.

**Diluent:** Isopropyl alcohol, water, and phosphoric acid (40: 60: 0.1)

**Mobile phase:** Acetonitrile and *Buffer* (45:55)

**Standard solution:** 0.04 mg/mL of USP Bzotroprine Mesylate RS in *Diluent*

**Sample solution:** Nominally 0.04 mg/mL of bztroprine mesylate from a suitable amount of powdered Tablets in *Diluent* prepared as follows. Add a suitable amount of fine powder from NLT 20 Tablets to a portion of *Diluent* corresponding to 60% of the final volume. Mix by mechanical means for NLT 60 min, and dilute with *Diluent* to volume. Centrifuge a portion of this mixture, and filter the supernatant layer.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 259 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 0.7 mL/min

**Injection volume:** 50 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 4.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bztroprine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bzotroprine Mesylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bztroprine mesylate in the *Sample solution* (mg/mL)

**Bzotroprine Mesylate Tablets****DEFINITION**

Bzotroprine Mesylate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bztroprine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ).

**IDENTIFICATION****• A.**

**Standard stock solution:** 0.2 mg/mL of USP Bzotroprine Mesylate RS

**Standard solution:** In a separator containing the *Standard stock solution* add 2 mL of 1 N sodium hydroxide. Extract with three 10-mL portions of chloroform, collecting the chloroform extracts to a 50-mL beaker. Evaporate the chloroform extracts with the aid of gentle heat and a current of air to dryness, and dissolve the residue in 1 mL of chloroform.

**Sample stock solution:** Dissolve a portion of finely powdered Tablets, equivalent to 10 mg of bztroprine



Acceptance criteria: 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Determine the amount of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ) dissolved by using the following method.

Buffer: Transfer 0.83 mL of octylamine to a 1-L volumetric flask, dilute to volume, and adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Acetonitrile and Buffer (65:35)

Standard solution: USP Benztropine Mesylate RS in Medium. Dilute to obtain a solution having a known concentration similar to that of the Sample solution.

Sample solution: Use a filtered portion of the solution under test from the dissolution vessel.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm  $\times$  25-cm; packing L7

Flow rate: 2 mL/min

Injection volume: 300  $\mu$ L

#### System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 3.0%

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Benztropine Mesylate RS in the Standard solution (mg/mL)

$V$  = volume of the Medium, 900 mL

$L$  = label claim (mg/Tablet)

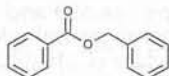
Acceptance criteria: NLT 80% (Q) of the labeled amount of benztropine mesylate ( $C_{21}H_{25}NO \cdot CH_4O_3S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
USP Benztropine Mesylate RS

## Benzyl Benzoate



$C_{14}H_{12}O_2$   
Benzoic acid, phenylmethyl ester;  
Benzyl benzoate [120-51-4].

212.24

### DEFINITION

Benzyl Benzoate contains NLT 99.0% and NMT 100.5% of  $C_{14}H_{12}O_2$ .

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197F)**

### ASSAY

#### • PROCEDURE

Sample: 2 g of Benzyl Benzoate

Analysis: Transfer the Sample to a conical flask fitted with a reflux condenser. Add 50.0 mL of 0.5 N alcoholic potassium hydroxide VS, and boil gently for 1 h. Cool, add phenolphthalein TS, and titrate with 0.5 N hydrochloric acid VS. Perform a blank determination (see *Titrimetry* (541)). Each mL of 0.5 N alcoholic potassium hydroxide is equivalent to 106.1 mg of Benzyl Benzoate ( $C_{14}H_{12}O_2$ ).

Acceptance criteria: 99.0%–100.5%

### IMPURITIES

#### • LIMIT OF ALDEHYDES

Sample solution: Transfer 10.0 g to a 125-mL conical flask containing 50 mL of alcohol and 5 mL of hydroxylamine hydrochloride solution (3.5 in 100), mix, and allow to stand for 10 min.

Analysis: Add 1 mL of bromophenol blue TS, and titrate with 0.1 N sodium hydroxide VS to a light green endpoint. Perform a blank determination, and match the color of the endpoint with that of the titrated Sample solution.

Acceptance criteria: The net volume of 0.1 N sodium hydroxide consumed does not exceed 0.50 mL (0.05% as benzaldehyde).

### SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 1.116–1.120
- **CONGEALING TEMPERATURE (651):** Congelation may be brought about by the addition of a fragment of previously congealed Benzyl Benzoate when the temperature has reached the expected congealing temperature.  
Acceptance criteria: NLT 18.0°
- **REFRACTIVE INDEX (831):** 1.568–1.570 at 20°
- **ACIDITY:** Add 2 drops of phenolphthalein TS to 25 mL of alcohol, and add 0.020 N sodium hydroxide until a pink color is produced. Add 5.0 g of Benzyl Benzoate, and titrate with 0.020 N sodium hydroxide.  
Acceptance criteria: NMT 1.5 mL of 0.020 N sodium hydroxide is required to restore the pink color.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, well-filled, light-resistant containers, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS (11)**  
USP Benzyl Benzoate RS

## Benzyl Benzoate Lotion

### DEFINITION

Benzyl Benzoate Lotion contains NLT 26.0% and NMT 30.0% (w/w) of benzyl benzoate ( $C_{14}H_{12}O_2$ ).

Benzyl Benzoate	250 mL
Triethanolamine	5 g
Oleic Acid	20 g
Purified Water	750 mL
To make about	1000 mL

Mix the Triethanolamine with the Oleic Acid, add the Benzyl Benzoate, and mix. Transfer the mixture to a suitable container of 2000-mL capacity, add 250 mL of Purified Water, and shake the mixture thoroughly. Add the remaining Purified Water, and again shake thoroughly.



**ASSAY****• PROCEDURE**

**Sample:** 5 g of Lotion, accurately weighed, in a conical flask

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Residual titration

**Titrant:** 0.1 N sodium hydroxide VS

**Back-titrant:** 0.5 N hydrochloric acid VS

**Endpoint detection:** Visual

**Analysis:** To the *Sample* add 25 mL of alcohol and 2 drops of phenolphthalein TS. Cool the solution to 15°, and titrate quickly with *Titrant* to a slight pink color. Add 50.0 mL of 0.5 N alcoholic potassium hydroxide VS, connect the flask to a reflux condenser, and boil gently for 1 h. Cool. Promptly add phenolphthalein TS and titrate with *Back-titrant*. Perform a blank determination. Each mL of 0.5 N alcoholic potassium hydroxide is equivalent to 106.1 mg of benzyl benzoate ( $C_{14}H_{12}O_2$ ).

**Acceptance criteria:** 26.0%–30.0% (w/w)

**SPECIFIC TESTS**

**• pH (791):** 8.5–9.2

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Package in tight containers.

## Benzylpenicilloyl Polylysine Concentrate

» Benzylpenicilloyl Polylysine Concentrate has a molar concentration of benzylpenicilloyl moiety ( $C_{16}H_{19}N_2O_5S$ ) of not less than 0.0125 M and not more than 0.020 M. It contains one or more suitable buffers.

**Packaging and storage—**Preserve in tight containers.

**Labeling—**The label states that this article is not intended for direct administration to humans or animals.

**USP Reference standards (11)—**

USP L-Lysine Hydrochloride RS

**pH (791):** between 6.5 and 8.5, the undiluted Concentrate being used.

**Limit of penicillenate and penamaldate—**Transfer 1 mL of Concentrate to a 50-mL volumetric flask, dilute with *Saline phosphate buffer*, prepared as directed in the *Assay*, to volume, and mix. Using a suitable spectrophotometer and using *Saline phosphate buffer* as a blank, determine the absorbances at the wavelengths of maximum absorption at about 322 nm and 282 nm. Calculate the molar concentration of penicillenate taken by the formula:

$$50A_{322} / 26,600b$$

in which  $A_{322}$  is the absorbance at 322 nm, 26,600 is the molar absorptivity of the penicillenate moiety at pH 7.6, and  $b$  is the length of the cell, in cm: not more than 0.00020 M is found. Calculate the molar concentration of penamaldate taken by the formula:

$$50A_{282} / 22,325b$$

in which  $A_{282}$  is the absorbance at 282 nm, 22,325 is the molar absorptivity of the penamaldate moiety at pH 7.6, and  $b$  is the length of the cell, in cm: not more than 0.00060 M is found.

**Benzylpenicilloyl substitution—**

**Citrate buffer—**Dissolve 19.69 g of sodium citrate dihydrate, 0.1 mL of pentachlorophenol, and 5 mL of 2,2'-thiodiethanol in 900 mL of 0.2 N hydrochloric acid, adjust with hydrochloric acid to a pH of 2.2, dilute with water to 1000 mL, and mix.

**Ninhydrin reagent—**Dissolve 18 g of ninhydrin and 0.7 g of hydrindantin in 675 mL of dimethyl sulfoxide, add 225 mL of 4 M lithium acetate solution previously adjusted with glacial acetic acid to a pH of 5.2, and mix.

**Standard preparation—**Dissolve an accurately weighed quantity of USP L-Lysine Hydrochloride RS in *Citrate buffer* to obtain a solution having a known concentration of about 91 µg per mL ( $5 \times 10^{-4}$  M).

**Test preparation—**Transfer 1.0 mL of Concentrate to a 10-mL volumetric flask, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to an ampul, add 1.5 mL of 6 N hydrochloric acid, and seal the ampul under nitrogen. Heat the ampul at 110° for 22 hours. Transfer the contents of the ampul to a round-bottom, 50-mL flask, and dry by vacuum rotary evaporation. Dissolve the residue three times, using 5-mL portions of water, evaporating to dryness after each dissolution. Dissolve the residue in 10 mL of *Citrate buffer*.

**Chromatographic system (see *Chromatography* (621))—**The liquid chromatograph is equipped with a 1.75-mm × 50-cm column that contains a packing of 8-µm 8% cross-linked sulfonated divinylbenzene polystyrene cation-exchange resin. The column effluent is mixed continuously with flowing *Ninhydrin reagent*, and the flowing mixture is heated at 130° for 1.5 minutes in a reaction coil. The absorbance of the reaction mixture is measured continuously by a 570-nm detector. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*; the column efficiency determined from the analyte peak is not less than 1800 theoretical plates, and the relative standard deviation for replicate injections is not more than 4.0%.

**Procedure—**Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The retention time is about 57 minutes for L-lysine. Calculate the molar concentration of lysine in the Concentrate taken by the formula:

$$(0.1C/182.65)(r_U / r_S)$$

in which  $C$  is the concentration, in µg per mL, of USP L-Lysine Hydrochloride RS in the *Standard preparation*; 182.65 is the molecular weight of anhydrous lysine hydrochloride; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively. Calculate the percentage of benzylpenicilloyl substitution taken by the formula:

$$100(B/L)$$

in which  $B$  is the molar concentration of benzylpenicilloyl moiety in the Concentrate, as determined in the *Assay*; and  $L$  is the molar concentration of lysine in the Concentrate: not less than 50% and not more than 70% is found.

**Assay—**

**Saline phosphate buffer—**Dissolve 9 g of sodium chloride and 1.38 g of monobasic sodium phosphate in 900 mL of water, adjust with 5 N sodium hydroxide or phosphoric acid to a pH of 7.6, dilute with water to 1000 mL, and mix.

**Mercuric chloride solution—**Dissolve 35 mg of mercuric chloride in 500 mL of water, and mix.

**Assay preparation—**Transfer 1.0 mL of Concentrate to a 500-mL volumetric flask, dilute with *Saline phosphate buffer* to volume, and mix.

**Procedure—**Transfer 3.0 mL of *Assay preparation* to a spectrophotometric cell. Using a suitable spectrophotometer and



using *Saline phosphate buffer* as the blank, determine the initial absorbance at the wavelength of maximum absorbance at about 282 nm. Add 0.02 mL of *Mercuric chloride solution* to the *Assay preparation* in the spectrophotometric cell, mix, and determine the absorbance at the same wavelength after 1 and 3 minutes. Repeat the addition of 0.02-mL portions of *Mercuric chloride solution* until a maximum absorbance reading is obtained. Calculate the molar concentration of benzylpenicilloyl moiety in the Concentrate taken by the formula:

$$500\{[A_m(3 + 0.02n)/3] - A_i\}/22,325b$$

in which  $A_m$  is the highest absorbance observed;  $A_i$  is the initial absorbance,  $n$  is the number of 0.02-mL portions of *Mercuric chloride solution* added to the *Assay preparation* to obtain the maximum absorbance; 22,325 is the molar absorptivity of the penamaldate formed by the reaction of benzylpenicilloyl with mercuric chloride at pH 7.6; and  $b$  is the length of the cell, in cm: between 0.0125 M and 0.020 M is found.

## Benzylpenicilloyl Polylysine Injection

» Benzylpenicilloyl Polylysine Injection has a molar concentration of benzylpenicilloyl moiety ( $C_{16}N_2H_{19}O_5S$ ) of not less than  $5.4 \times 10^{-5}$  M and not more than  $7.0 \times 10^{-5}$  M. It contains one or more suitable buffers.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, in a refrigerator.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Bacterial Endotoxins Test** (85)—It contains not more than 5833.0 USP Endotoxin Units per mL.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 6.5 and 8.5.

**Assay**—

*Saline phosphate buffer* and *Mercuric chloride solution*—Prepare as directed in the Assay under *Benzylpenicilloyl Polylysine Concentrate*.

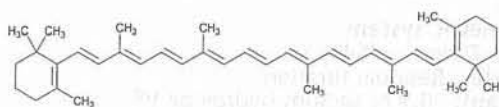
*Assay preparation*—Combine the contents of a sufficient number of containers to obtain not less than 3 mL of Injection. Transfer 3.0 mL of Injection to a 10-mL volumetric flask, dilute with *Saline phosphate buffer* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under *Benzylpenicilloyl Polylysine Concentrate*. Calculate the molar concentration of benzylpenicilloyl moiety in the Injection taken by the formula:

$$(10 / 3)\{[A_m(3 + 0.02n) / 3] - A_i\} / 22,325b$$

in which the terms are as defined therein.

## Beta Carotene



$C_{40}H_{56}$

536.87

$\beta$ , $\beta$ -Carotene;  
all-*trans*- $\beta$ -Carotene;  
(all-*E*)-1,1'-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene] [7235-40-7].

### DEFINITION

Beta Carotene contains NLT 96.0% and NMT 101.0% of total carotenoids calculated as beta carotene ( $C_{40}H_{56}$ ). It contains NLT 95% of all-*trans*-beta carotene in the total carotenoids content.

### IDENTIFICATION

#### A.

**Sample solution:** Prepare as directed in the *Sample solution* in the test for *Content of Total Carotenoids*.

**Analysis:** Record the UV-Vis spectrum from 300–600 nm.

**Acceptance criteria:** The *Sample solution* shows a shoulder at about 427 nm, an absorption maximum at about 455 nm, and another maximum at about 483 nm. The absorbance ratio  $A_{455}/A_{483}$  is between 1.14 and 1.18.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Content of Beta Carotene*.

### COMPOSITION

#### • CONTENT OF TOTAL CAROTENOIDS

[NOTE—Use low-actinic glassware.]

**Sample stock solution:** 0.1 mg/mL of Beta Carotene in tetrahydrofuran

**Sample solution:** Transfer 3.0 mL of *Sample stock solution* to a 100-mL volumetric flask, and dilute with cyclohexane to volume.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Analytical wavelength:** 456 nm

**Cell path:** 1 cm

**Blank:** Cyclohexane

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of total carotenoids ( $T$ ) as beta carotene ( $C_{40}H_{56}$ ):

$$T = A/(F \times C)$$

$A$  = absorbance of the *Sample solution*  
 $F$  = 2505, coefficient of extinction ( $E^{1\%}$ ) of pure all-*trans*-beta carotene in cyclohexane (100 mL · g<sup>-1</sup> · cm<sup>-1</sup>)

$C$  = concentration of the *Sample solution* (g/mL)

**Acceptance criteria:** 96.0%–101.0% of total carotenoids as beta carotene ( $C_{40}H_{56}$ )

#### • CONTENT OF BETA CAROTENE

[NOTE—Use low-actinic glassware.]

**Mobile phase:** Transfer 50 mg of butylated hydroxytoluene to a 1-L volumetric flask, and dissolve with 20 mL of 2-propanol. Add 0.2 mL of *N*-ethyl-diisopropylamine, 25 mL of 0.2% ammonium acetate solution, 455 mL of acetonitrile, and about 450 mL of methanol. Allow the solution to reach room temperature, and dilute with methanol to volume.

**Diluent:** 50 µg/mL of butylated hydroxytoluene in alcohol



**System suitability solution:** Transfer 20 mg of USP Beta Carotene System Suitability RS to a 50-mL volumetric flask. Add 1 mL of water and 4 mL of tetrahydrofuran, and sonicate for 5 min. Dilute with *Diluent* to volume, and sonicate for 5 min. Cool to room temperature, pass the suspension through a membrane filter of 0.45- $\mu$ m pore size, and use the clear filtrate.

**Standard solution:** 10  $\mu$ g/mL of USP Beta Carotene RS in tetrahydrofuran and *Diluent* (1:9). Dissolve an appropriate amount of USP Beta Carotene RS in a volumetric flask first with tetrahydrofuran, using 10% of the volume of the flask, then dilute with *Diluent* to volume.

**Sample solution:** Dilute the freshly prepared *Sample stock solution* as prepared in the test for *Content of Total Carotenoids* (1 in 10) with *Diluent*.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 448 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L68

**Column temperature:** 30°

**Flow rate:** 0.6 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

The approximate relative retention times of the components in the *System suitability solution* are listed in *Table 1*.

**Table 1**

Analyte	Relative Retention Time	Relative Response Factor
all- <i>trans</i> -Alpha carotene	0.93	1.0
all- <i>trans</i> -Beta carotene	1.00	1.0
9- <i>cis</i> -Beta carotene	1.07	1.0
13- <i>cis</i> -Beta carotene	1.17	1.2
15- <i>cis</i> -Beta carotene	1.21	1.4

#### Suitability requirements

**Chromatogram similarity:** The chromatogram from the *System suitability solution* is similar to the reference chromatogram provided with the lot of USP Beta Carotene System Suitability RS being used.

**Resolution:** NLT 1.5 between all-*trans*-beta carotene and all-*trans*-alpha carotene; NLT 1.2 between all-*trans*-beta carotene and 9-*cis*-beta carotene, *System suitability solution*

**Tailing factor:** NMT 2.0 for the all-*trans*-beta carotene peak, *Standard solution*

**Relative standard deviation:** NMT 2.0% for the all-*trans*-beta carotene peak from replicate injections, *Standard solution*

#### Analysis

**Sample:** *Sample solution*

Record the chromatograms, and identify the peaks of the relevant analytes of the *Sample solution* by comparing with those of the *System suitability solution*. Measure the peak area responses.

Calculate the percentage of all-*trans*-beta carotene relative to total carotenoids in the sample taken:

$$\text{Result} = (r_u/r_T) \times 100$$

$r_u$  = peak area of all-*trans*-beta carotene from the *Sample solution*

$$r_T = [(\text{peak area of all-}i\text{trans-}\alpha \text{ carotene} \times 1.0) + (\text{peak area of all-}i\text{trans-}\beta \text{ carotene}) + (\text{peak area of 9-}i\text{cis-}\beta \text{ carotene}) + (\text{peak area of 13-}i\text{cis-}\beta \text{ carotene} \times 1.2) + (\text{peak area of 15-}i\text{cis-}\beta \text{ carotene} \times 1.4) + (\text{sum of peak areas of other } i\text{cis-isomers of beta carotene})]$$

from the *Sample solution*

**Acceptance criteria:** NLT 95% of all-*trans*-beta carotene in the total carotenoids content

#### • ALPHA CAROTENE AND OTHER RELATED COMPOUNDS

**Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the test for *Content of Beta Carotene*.

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of alpha carotene and other individual related compounds relative to total carotenoids in the portion of the *Sample* taken:

$$\text{Result} = (r_u/r_T) \times 100$$

$$r_u = (\text{peak area of all-}i\text{trans-}\alpha \text{ carotene} \times 1.0) \text{ or } (\text{peak area response of other individual related compounds} \times \text{appropriate relative response factor, Table 1}) \text{ in the } i\text{Sample solution}$$

$$r_T = [(\text{peak area of all-}i\text{trans-}\alpha \text{ carotene} \times 1.0) + (\text{peak area of all-}i\text{trans-}\beta \text{ carotene}) + (\text{peak area of 9-}i\text{cis-}\beta \text{ carotene}) + (\text{peak area of 13-}i\text{cis-}\beta \text{ carotene} \times 1.2) + (\text{peak area of 15-}i\text{cis-}\beta \text{ carotene} \times 1.4) + (\text{sum of peak areas of other } i\text{cis-isomers of beta carotene})]$$

from the *Sample solution*

#### Acceptance criteria

**Alpha carotene:** NMT 1.0%

**Total related compounds (including alpha carotene):** NMT 5%

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%, 2 g of specimen being used

#### Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1-Jan-2018)

#### SPECIFIC TESTS

- **LOSS ON DRYING** (731)

**Analysis:** Dry under vacuum over phosphorus pentoxide at 40° for 4 h.

**Acceptance criteria:** NMT 0.2%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- **USP REFERENCE STANDARDS** (11)

USP Beta Carotene RS

(all-*E*)-1,1'-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene].

USP Beta Carotene System Suitability RS

## Beta Carotene Capsules

#### DEFINITION

Beta Carotene Capsules contain NLT 90% and NMT 125.0% of the labeled amount of total beta carotene (C<sub>40</sub>H<sub>56</sub>), of which NLT 95.0% is the all-*trans*-beta carotene isomer.

(Postponed indefinitely)



## IDENTIFICATION

## • A.

**Sample solution:** Dilute the *Sample stock solution* of the test for *Content of Total Beta Carotene* with cyclohexane to a final concentration of between 1 and 5 µg/mL of beta carotene. Pass through a membrane filter of 0.45-µm pore size.

**Analysis:** Record the UV-Vis spectrum from 300 to 600 nm.

**Acceptance criteria:** The *Sample solution* shows a shoulder at about 427 nm, an absorption maximum at about 455 nm, and another maximum at about 483 nm. The absorbance ratio  $A_{455}/A_{483}$  is between 1.14 and 1.18.

- B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Content of Total Beta Carotene*.

## ASSAY

## • CONTENT OF TOTAL BETA CAROTENE

[NOTE—Use low-actinic glassware.]

**Mobile phase:** Transfer 50 mg of butylated hydroxytoluene into a 1-L volumetric flask, and dissolve with 20 mL of 2-propanol. Add 0.2 mL of *N*-ethyl-diisopropylamine, 25 mL of 0.2% ammonium acetate solution, 455 mL of acetonitrile, and about 450 mL of methanol. Allow the solution to reach room temperature, and dilute with methanol to volume.

**Diluent:** 50 mg/L of butylated hydroxytoluene in alcohol

**System suitability solution:** Transfer 20 mg of USP Beta Carotene System Suitability RS to a 50-mL volumetric flask. Add 1 mL of water and 4 mL of tetrahydrofuran, and sonicate for 5 min. Dilute with *Diluent* to volume, and sonicate for 5 min. Cool to room temperature, pass through a membrane filter of 0.45-µm pore size, and use the clear filtrate.

**Standard stock solution:** 60 µg/mL of USP Beta Carotene RS in tetrahydrofuran. [NOTE—The USP Beta Carotene RS is subjected to the spectrophotometric purity test at the time of analysis; see the determination of the concentration of *Standard solution A* below.]

**Standard solution A:** Transfer 5.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 5.0 mL of tetrahydrofuran, and dilute with *Diluent* to volume.

Determine the concentration of *Standard solution A* according to the *Analysis of Standard solution B*. [NOTE—The concentration of *Standard solution B* equals the concentration of *Standard solution A*.]

**Standard solution B:** Transfer 5.0 mL of the *Standard stock solution* into a 100-mL volumetric flask, and dilute with cyclohexane to volume. Prepare in triplicate.

## Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Analytical wavelength:** 457 nm

**Cell:** 1 cm

**Blank:** Cyclohexane

## Analysis

**Sample:** *Standard solution B*

Calculate the concentration of total beta carotene (µg/mL) as all-*trans*-beta carotene ( $C_{40}H_{56}$ ) in *Standard solution B*:

$$\text{Result} = (A_U/a) \times F$$

$A_U$  = average absorbance of the three preparations of *Standard solution B*

$a$  = absorptivity of pure all-*trans*-beta carotene in cyclohexane at 457 nm ( $\text{mL} \cdot \text{mg}^{-1} \cdot \text{cm}^{-1}$ ), 250

$F$  = conversion factor from mg to µg (µg/mg), 1000

**Sample stock solution:** Randomly select a number of Capsules, equivalent to 10–50 mg of beta carotene,

with a total weight not exceeding 5 g. For powder-containing Capsules, empty the shell, and transfer shell and contents into a 250-mL volumetric flask. For Capsules containing liquid formulations, place the Capsules directly into a 250-mL volumetric flask. Add 250 mg of butylated hydroxytoluene, 0.5 mL of alkaline protease R, and 15 mL of water. Swirl the flask gently to wet the entire contents. Sonicate the flask at 50° for 30 min, and swirl the flask every 10 min. Add 100 mL of alcohol to the warm suspension, and shake vigorously. Add 110 mL of methylene chloride, and shake vigorously again. Disperse any clumps with homogenizer, and rinse the homogenizer probe with 15 mL of methylene chloride into the flask. Allow the solution to stand in the dark until it reaches room temperature (about 2 h), dilute with methylene chloride to volume, shake vigorously, and allow the solids to settle.

**Sample solution:** Dilute a volume of the *Sample stock solution* with a *Diluent*–methylene chloride mixture (1:1) so that the final concentration of beta carotene is between 1 and 5 µg/mL. Pass through a membrane filter of 0.45-µm pore size.

## Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 448 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L68

**Column temperature:** 30°

**Flow rate:** 0.6 mL/min

**Injection volume:** 20 µL

## System suitability

**Samples:** *System suitability solution* and *Standard solution A*

[NOTE—The approximate relative retention times of the components in the *System suitability solution* are listed in *Table 1*.]

Table 1

Name	Relative Retention Time	Relative Response Factor
all- <i>trans</i> -Alpha carotene	0.93	1.1
all- <i>trans</i> -Beta carotene	1.00	1
9- <i>cis</i> -Beta carotene	1.07	1
13- <i>cis</i> -Beta carotene	1.17	1.2
15- <i>cis</i> -Beta carotene	1.21	1.4

## Suitability requirements

**Chromatogram similarity:** The chromatogram from the *System suitability solution* is similar to the reference chromatogram provided with the lot of USP Beta Carotene System Suitability RS being used.

**Resolution:** NLT 1.5 between all-*trans*-beta carotene and all-*trans*-alpha carotene and between all-*trans*-beta carotene and 9-*cis*-beta carotene, *System suitability solution*

**Tailing factor:** NMT 2.0 for the all-*trans*-beta carotene peak, *Standard solution A*

**Relative standard deviation:** NMT 2.0% for the all-*trans*-beta carotene peak from replicate injections, *Standard solution A*

## Analysis

**Samples:** *Standard solution A* and *Sample solution*  
Identify the peaks of the relevant analytes of the *Sample solution* by comparing with those of the *System suitability solution*. Measure the peak area responses.

Calculate the percentage of the labeled amount of total beta carotene in the Capsules taken:

$$\text{Result} = (\Sigma r_U/r_S) \times (C_S/C_U) \times 100$$



$\Sigma r_U$  = [(peak area of all-*trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene  $\times$  1.2) + (peak area of 15-*cis*-beta carotene  $\times$  1.4) from the *Sample solution*]

$r_s$  = peak area of all-*trans*-beta carotene from *Standard solution A*

$C_s$  = concentration of all-*trans*-beta carotene in *Standard solution A* as determined above ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of total beta carotene in the *Sample solution* ( $\mu\text{g/mL}$ )

Calculate the percentage of all-*trans*-beta carotene in the portion of Capsules taken:

$$\text{Result} = (r_{\text{all-trans}}/\Sigma r_U) \times 100$$

$r_{\text{all-trans}}$  = peak area of all-*trans*-beta carotene from the *Sample solution*

$\Sigma r_U$  = [(peak area of all-*trans*-beta carotene) + (peak area of 9-*cis*-beta carotene) + (peak area of 13-*cis*-beta carotene  $\times$  1.2) + (peak area of 15-*cis*-beta carotene  $\times$  1.4) from the *Sample solution*]

**Acceptance criteria:** 90%–125.0% of the labeled amount of total beta carotene ( $\text{C}_{40}\text{H}_{56}$ ), of which NLT 95.0% is the all-*trans*-beta carotene isomer  
(Postponed indefinitely)

## SPECIFIC TESTS

### • ALPHA CAROTENE AND OTHER RELATED COMPOUNDS

**Mobile phase, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the test for *Content of Total Beta Carotene*.

**Analysis**

**Sample:** *Sample solution*

**Injection volume:** 20  $\mu\text{L}$

Calculate the percentage of alpha carotene and other individual related compounds relative to total beta carotene in the portion of Capsules taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak area of alpha carotene or other individual related compounds

$r_T$  = sum of the areas of all the peaks

**Acceptance criteria**

**Alpha carotene:** NMT 1.0%

**Total related compounds (including alpha carotene):**  
NMT 5.0%

## PERFORMANCE TESTS

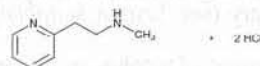
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** The label states the name and content of any carriers and antioxidants added to the formulation and the content of total carotenoids as beta carotene.
- **USP REFERENCE STANDARDS (11)**  
USP Beta Carotene RS  
(all-*E*)-1,1'-(3,7,12,16-Tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene].  
 $\text{C}_{40}\text{H}_{56}$  536.87

## USP Beta Carotene System Suitability RS

## Betahistine Hydrochloride



$\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{HCl}$  209.12

2-Pyridineethanamine, *N*-methyl-, dihydrochloride.

2-[2-(Methylamino)ethyl]pyridine dihydrochloride  
[5579-84-0].

» Betahistine Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of  $\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{HCl}$ , calculated on the dried basis.

### USP Reference standards (11)—

USP Betahistine Hydrochloride RS

### Identification—

**A: Infrared Absorption (197K).**

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**pH (791):** between 2.0 and 3.0, in a solution (1 in 10).

**Loss on drying (731)—**Dry it between 100° and 105° to constant weight: it loses not more than 1.0% of its weight.

**Residue on ignition (281):** not more than 0.1%.

### Related compounds—

**Mobile phase and Chromatographic system—**Proceed as directed in the *Assay*.

**Test solution—**Transfer about 38 mg of Betahistine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Procedure—**Inject about 10  $\mu\text{L}$  of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Betahistine Hydrochloride taken by the formula:

$$100F(r_i/r_s)$$

in which *F* is the response factor of the respective impurity (see *Table 1*) and 1.0 for all other peaks;  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all of the peaks, adjusted for the relative response factor.

Table 1

Impurity Name	Relative Retention Time	Response Factor ( <i>F</i> )	Limit (%)
2-(2-Hydroxyethyl)pyridine	0.3	0.5	0.2
2-Vinylpyridine	0.4	0.4	0.2
<i>N</i> -Methyl- <i>N</i> , <i>N</i> -bis(2-pyridin-2-yl-ethyl)-amine	2.4	1.4	0.2

In addition to not exceeding the limits for impurities in *Table 1*, not more than 0.1% of any other individual impurity is found; and not more than 0.5 % of total impurities is found.



**Assay—**

**Ammonium acetate buffer**—Dissolve about 0.69 g of ammonium acetate in 1000 mL of water. Adjust with glacial acetic acid to a pH of 4.7.

**Mobile phase**—Prepare a filtered and degassed mixture of 350 mL of acetonitrile and 650 mL of **Ammonium acetate buffer**, containing 2.88 g of sodium lauryl sulfate. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betahistine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.38 mg per mL.

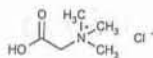
**Assay preparation**—Transfer about 38 mg of Betahistine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and 3.0-mm × 15-cm column that contains packing L1. The column temperature is maintained at 40°. The flow rate is about 0.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 5000 theoretical plates; the tailing factor for the betahistine peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_8H_{12}N_2 \cdot 2HCl$  in the portion of Betahistine Hydrochloride taken by the formula:

$$100C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Betahistine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Betaine Hydrochloride**

$C_5H_{11}NO_2 \cdot HCl$  153.61  
Methanaminium, 1-carboxy-N, N, N-trimethyl-, chloride.  
Betaine hydrochloride.  
(Carboxymethyl)trimethylammonium chloride [590-46-5].

» Betaine Hydrochloride contains not less than 98.0 percent and not more than 100.5 percent of  $C_5H_{11}NO_2 \cdot HCl$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Betaine Hydrochloride RS

**Identification—**

A: *Infrared Absorption* (197K).

B: A solution (1 in 20) responds to the tests for *Chloride* (191).

pH (791): between 0.8 and 1.2, in a solution (1 in 4).

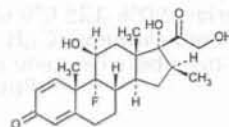
**Water Determination, Method I** (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.1%.

**Delete the following:**

• **Heavy metals** (231): 0.001%. (Official 1-Jan-2018)

**Assay**—Transfer about 400 mg of Betaine Hydrochloride, accurately weighed, to a conical flask, add 50 mL of glacial acetic acid, and heat gently with swirling until solution is complete. Add 25 mL of mercuric acetate TS, cool, add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 15.36 mg of  $C_5H_{11}NO_2 \cdot HCl$ .

**Betamethasone**

$C_{22}H_{29}FO_5$  392.46

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17,21-trihydroxy-16-methyl-, (11β,16β)-.

9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione [378-44-9].

» Betamethasone contains not less than 97.0 percent and not more than 103.0 percent of  $C_{22}H_{29}FO_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers. Store between 2° and 30°.

**USP Reference standards** (11)—

USP Betamethasone RS

**Identification—**

A: *Infrared Absorption* (197M).

B: *Thin-Layer Chromatographic Identification Test* (201)—

**Test solution**—Prepare a solution of Betamethasone in dehydrated alcohol containing 0.5 mg per mL.

**Developing solvent system**: a mixture of chloroform and diethylamine (2:1).

**Procedure**—Proceed as directed in the chapter, except to locate the spots by lightly spraying with dilute sulfuric acid (1 in 2) and heating on a hot plate or under a lamp until spots appear.

**Specific rotation** (781S): between +118° and +126°, calculated on the dried basis.

**Test solution**: 5 mg per mL, in methanol.

**Loss on drying** (731)—Dry it at 105° for 3 hours; it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%, a platinum crucible being used.



**Ordinary impurities** (466)—

*Test solution:* methanol.

*Standard solution:* methanol.

*Application volume:* 10  $\mu$ L.

*Eluant:* a mixture of toluene, acetone, methyl ethyl ketone, and formic acid (55:20:20:5), in a nonequilibrated chamber.

*Visualization:* 5.

**Assay—**

*Mobile phase*—Prepare a filtered and degassed mixture of water and acetonitrile (63:37). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Prepare a solution of propylparaben in alcohol having a known concentration of about 0.25 mg per mL.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Betamethasone RS in alcohol to obtain a solution having a known concentration of about 0.2 mg per mL. Transfer 10.0 mL of this solution to a suitable vial, and add 10.0 mL of *Internal standard solution*, to obtain a *Standard preparation* having known concentrations of about 0.1 mg of betamethasone and about 0.125 mg of propylparaben per mL.

*Assay preparation*—Using about 80 mg of Betamethasone, accurately weighed, prepare as directed for *Standard preparation*.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for betamethasone and 1.4 for propylparaben; the resolution,  $R$ , between betamethasone and propylparaben is not less than 3.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in the portion of Betamethasone taken by the formula:

$$800C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Betamethasone RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak height ratios of the betamethasone peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Betamethasone Cream****DEFINITION**

Betamethasone Cream contains NLT 90.0% and NMT 115.0% of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ) in a suitable cream base.

**IDENTIFICATION**

• **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

*Standard solution:* 1 mg/mL of USP Betamethasone RS in dehydrated alcohol

*Sample solution:* Nominally 1 mg/mL of betamethasone prepared by concentrating 10 mL of the *Sample solution* from the *Assay* on a steam bath to 1 mL

*Chromatographic system*

*Developing solvent system:* Chloroform and diethylamine (2:1)

*Spray reagent:* Methanol, sulfuric acid, and nitric acid (10:10:1)

**Analysis**

*Samples:* *Standard solution* and *Sample solution*

Proceed as directed in the chapter, except spray the plate with the *Spray reagent*, and heat at 105° for 10 min.

**Acceptance criteria:** Meets the requirements

**ASSAY**

• **PROCEDURE**

*Mobile phase:* Acetonitrile and water (37:63)

*Internal standard solution:* 0.25 mg/mL of propylparaben in alcohol

*Standard stock solution:* 0.2 mg/mL of USP Betamethasone RS in alcohol

*Standard solution:* 0.1 mg/mL of USP Betamethasone RS prepared by combining 10.0 mL of the *Internal standard solution* and 10.0 mL of the *Standard stock solution*

*Sample solution:* Nominally 0.1 mg/mL of betamethasone prepared as follows. To a portion of Cream, nominally equivalent to 2 mg of betamethasone, add 10.0 mL of *Internal standard solution* and 10.0 mL of alcohol. Mix by rotation for 20 min. Centrifuge at 2500 rpm for 10 min. Transfer a portion of the supernatant to a suitable vial.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

*Mode:* LC

*Detector:* UV 240 nm

*Column:* 4.6-mm  $\times$  25-cm; packing L1

*Flow rate:* 1 mL/min

*Injection volume:* 10  $\mu$ L

**System suitability**

*Sample:* *Standard solution*

[NOTE—The relative retention times for betamethasone and propylparaben are 1.0 and 1.4, respectively.]

**Suitability requirements**

*Resolution:* NLT 3.0 between betamethasone and propylparaben

*Relative standard deviation:* NMT 2.0%

**Analysis**

*Samples:* *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Cream taken:

$$\text{Result} = (R_U / R_S) \times (C_S / C_U) \times 100$$

$R_U$  = peak height ratio of the betamethasone peak to the internal standard peak from the *Sample solution*

$R_S$  = peak height ratio of the betamethasone peak to the internal standard peak from the *Standard solution*

$C_S$  = concentration of USP Betamethasone RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–115.0%

**PERFORMANCE TESTS**

• **MINIMUM FILL** (755): Meets the requirements

**SPECIFIC TESTS**

• **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers.



- **USP REFERENCE STANDARDS** (11)  
USP Betamethasone RS

## Betamethasone Oral Solution

### DEFINITION

Betamethasone Oral Solution contains NLT 90.0% and NMT 115.0% of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ).

### IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Diluent:** Chloroform and methanol (1:1)

**Standard solution:** 1 mg/mL of USP Betamethasone RS in alcohol

**Sample solution:** Place a volume of Oral Solution, equivalent to about 1 mg of betamethasone, in a centrifuge tube. Add 15 mL of 0.1 N hydrochloric acid and 20 mL of ethyl acetate. Shake the tube for about 1 min. Centrifuge to separate the phases. Transfer the upper phase (ethyl acetate) to a suitable container. Evaporate to dryness on a steam bath under a gentle stream of nitrogen.

Allow to cool to room temperature. Dissolve the residue in about 0.5 mL of *Diluent* by using a vortex mixer. Transfer the solution to a 2-mL volumetric flask with small portions of *Diluent*. Dilute with *Diluent* to volume, and mix.

Evaporate 1 mL of the resulting solution on a steam bath just to dryness, and dissolve the residue in 0.5 mL of alcohol.

#### Chromatographic system

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Chloroform and diethylamine (2:1)

**Spray reagent:** Dilute sulfuric acid (1 in 2)

**Analysis:** Proceed as directed in the chapter. Locate the spots by lightly spraying with *Spray reagent*, and heat on a hot plate or under a lamp until spots appear.

**Acceptance criteria:** Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

Protect all standard and sample solutions from light.

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.9.

**Solution A:** Acetonitrile and *Buffer* (25:75)

**Solution B:** Acetonitrile and *Buffer* (45:55)

**Diluent:** Dehydrated alcohol and water (2:3)

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25.0	0	100
25.1	100	0
35.0	100	0

**Standard stock solution:** 0.12 mg/mL of USP Betamethasone RS prepared as follows. Transfer a quantity of USP Betamethasone RS to a suitable container, and dilute, using sonication, with dehydrated alcohol to obtain a solution containing 0.3 mg/mL. Quantitatively dilute an aliquot of this solution with water to obtain a 0.12-mg/mL solution of betamethasone.

**Standard solution:** 0.048 mg/mL of USP Betamethasone RS in *Diluent* from *Standard stock solution*

**Beclomethasone solution:** 0.12 mg/mL of beclomethasone prepared as follows. Transfer a quantity of beclomethasone to a suitable container, and dilute, using sonication, with dehydrated alcohol to obtain a solution containing 0.3 mg/mL. Quantitatively dilute an aliquot of this solution with water to obtain a 0.12 mg/mL solution of beclomethasone.

**System suitability solution:** 0.048 mg/mL each of USP Betamethasone RS and beclomethasone in *Diluent*, prepared from the *Standard stock solution* and *Beclomethasone solution*

**Sample solution:** Nominally 0.048 mg/mL of betamethasone prepared as follows. Transfer a measured volume of Oral Solution, containing a known amount of betamethasone, to a suitable volumetric flask, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 4- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for betamethasone and beclomethasone are 1.0 and 1.2, respectively.]

#### Suitability requirements

**Resolution:** NLT 4.0 between betamethasone and beclomethasone, *System suitability solution*

**Tailing factor:** NMT 1.5 for betamethasone, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Betamethasone RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–115.0%

### IMPURITIES

#### ORGANIC IMPURITIES

Protect all sample and standard solutions from light.

**Buffer, Solution A, Solution B, Diluent, Mobile phase, Standard stock solution, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.48  $\mu$ g/mL of USP Betamethasone RS in *Diluent* from the *Standard stock solution*

**Sensitivity solution:** 0.024  $\mu$ g/mL of USP

Betamethasone RS in *Diluent* from the *Standard solution*

#### System suitability

**Samples:** *System suitability solution* and *Sensitivity solution*

[NOTE—The relative retention times for betamethasone and beclomethasone are 1.0 and 1.2, respectively.]

#### Suitability requirements

**Resolution:** NLT 4.0 between betamethasone and beclomethasone, *System suitability solution*

**Relative standard deviation:** NMT 10% for betamethasone, *Sensitivity solution*



**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each related compound in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response for individual related compounds from the *Sample solution*  
 $r_S$  = peak response for betamethasone from the *Standard solution*  
 $C_S$  = concentration of USP Betamethasone RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of betamethasone in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** See Table 2.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone	1.0	—
9,11-Expoxy-17 $\alpha$ ,21-dihydroxy-16 $\beta$ -methylpregna-1,4 diene-3,20-dione	1.25	1.3
17 $\alpha$ ,21-Dihydroxy-16 $\beta$ -methylpregna-1,4,11-triene-3,20-dione	1.33	0.7

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the test for the absence of *Escherichia coli*. The total aerobic microbial count is NMT  $10^2$  cfu/mL, and the total combined molds and yeasts count is NMT  $10^1$  cfu/mL.
- **PH** (791): 2.8–3.6
- **DELIVERABLE VOLUME** (698): Meets the requirements for oral solution packaged in multiple-unit containers

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Store at controlled room temperature, protected from light. Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Betamethasone RS

**Betamethasone Tablets**

» Betamethasone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $\text{C}_{22}\text{H}_{29}\text{FO}_5$ ).

**Packaging and storage—**Preserve in tight containers. Store between 2° and 25°, excursions permitted between 15° and 30°. [NOTE—Protect the 21-tablet pack from excessive moisture.]

**USP Reference standards** (11)—

USP Betamethasone RS

**Identification—**Evaporate 50 mL of the *Assay preparation*, prepared as directed in the *Assay*, on a steam bath just to dryness, and dissolve the residue in 1 mL of chloroform. Proceed as directed for *Identification test B* under *Betamethasone*, beginning with "Apply 10  $\mu\text{L}$  of this solution."

**Dissolution, Procedure for a Pooled Sample** (711)—

**Medium:** water; 900 mL. Add 1.0 mL of *Internal standard solution* to each vessel.

**Apparatus 2:** 50 rpm.

**Time:** 45 minutes.

**Mobile phase—**Prepare a filtered and degassed mixture of methanol and water (60:40), making adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution—**Prepare a solution in methanol of testosterone having a final concentration of about 0.5 mg per mL.

**Standard solution—**Prepare a solution of USP Betamethasone RS, in methanol, having an accurately known concentration of about 0.5 mg per mL. Pipet 1 mL of this solution and 1 mL of the *Internal standard solution* into a container, and quantitatively dilute with water to 900 mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between betamethasone and testosterone is not less than 1.5; and the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure—**Separately inject equal volumes (about 200  $\mu\text{L}$ ) of the *Standard solution* and filtered portions of the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for betamethasone and 1.0 for testosterone. Calculate the quantity of  $\text{C}_{22}\text{H}_{29}\text{FO}_5$  dissolved in comparison with the *Standard solution*, similarly chromatographed.

**Tolerances—**Not less than 75% (Q) of the labeled amount of  $\text{C}_{22}\text{H}_{29}\text{FO}_5$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity—**

**Standard preparation—**Prepare as directed under *Assay for Steroids* (351), using USP Betamethasone RS, to obtain a solution having a known concentration of about 12  $\mu\text{g}$  per mL instead of 10  $\mu\text{g}$  per mL.

**Test preparation—**Weigh and finely powder 1 Tablet. Transfer to a 125-mL separator, add 20 mL of water, and shake. Extract the betamethasone completely, using three 15-mL portions of chloroform, filtering each extract through chloroform-washed cotton into a 50-mL volumetric flask. Dilute with chloroform to volume, and mix. Transfer 20.0 mL of this solution to a glass-stoppered, 50-mL conical flask, evaporate the chloroform on a steam bath just to dryness, cool, and dissolve the residue in 20.0 mL of alcohol.

**Procedure—**Proceed as directed under *Assay for Steroids* (351), except to keep the flasks in a constant-temperature bath at  $45 \pm 1^\circ$  for 90 minutes, then add 1.0 mL of glacial acetic acid, and cool. Calculate the quantity, in mg, of  $\text{C}_{22}\text{H}_{29}\text{FO}_5$  in the Tablet by the formula:

$$(TC/D)(A_U / A_S)$$

in which  $T$  is the labeled quantity, in mg, of betamethasone in the Tablet;  $C$  is the concentration, in  $\mu\text{g}$  per mL, of USP Betamethasone RS in the *Standard preparation*;  $D$  is the concentration, in  $\mu\text{g}$  per mL, of betamethasone in the *Test preparation*, based upon the labeled quantity per Tablet and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test preparation* and the *Standard preparation*, respectively.

**Assay—**

**Mobile phase—**Prepare a filtered and degassed mixture of water and acetonitrile (2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution—**Transfer about 25 mg of beclomethasone to a 200-mL volumetric flask, add methanol to volume, and mix.



**Standard preparation**—Dissolve an accurately weighed quantity of USP Betamethasone RS in methanol, and dilute quantitatively and stepwise, if necessary, with methanol to obtain a solution having a known concentration of about 0.1 mg per mL. Mix equal volumes, accurately measured, of this solution and the *Internal standard solution* to obtain a *Standard preparation* having a final known concentration of about 0.05 mg of USP Betamethasone RS per mL.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.5 mg of betamethasone, to a 125-mL separator. Add 25 mL of water, and shake by mechanical means for about 15 minutes. Add 5.0 mL of *Internal standard solution*. Extract with four 25-mL portions of chloroform. Filter the chloroform extracts through about 4 g of chloroform-washed anhydrous sodium sulfate, collecting the extracts in a 150-mL beaker. Evaporate the extracts on a steam bath with the aid of a stream of nitrogen to dryness, taking care to avoid overheating. Dissolve the residue in 2 mL of methanol, and transfer to a 10-mL volumetric flask. Rinse the beaker with small portions of methanol, transferring the rinses to the same flask. Dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak heights as directed for *Procedure*: the resolution,  $R$ , between the analyte and internal standard peaks is not less than 1.7; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the heights of the major peaks. The relative retention times are about 1.4 for beclomethasone and 1.0 for betamethasone. Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Tablets taken by the formula:

$$10C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Betamethasone RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak height ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Acetate

$C_{24}H_{31}FO_6$  434.50

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(acetyloxy)-, (11 $\beta$ ,16 $\beta$ )-.

9-Fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 21-acetate [987-24-6].

» Betamethasone Acetate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{24}H_{31}FO_6$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers. Store between 2° and 30°.

**USP Reference standards** (11)—

USP Betamethasone Acetate RS

**Identification**—

**A: Infrared Absorption** (197M).

**B: Thin-Layer Chromatographic Identification Test** (201)—

*Test solution*: 0.5 mg per mL in dehydrated alcohol.

*Developing solvent system*: a mixture of chloroform and diethylamine (2:1).

**Procedure**—Proceed as directed in the chapter. Locate the spots on the plate by lightly spraying with 10% sulfuric acid in alcohol and heating on a hot plate or under a lamp until spots appear.

**Specific rotation** (781S): between +120° and +128°.

*Test solution*: 10 mg per mL, in dioxane.

**Water Determination, Method I** (921): not more than 4.0%.

**Residue on ignition** (281): not more than 0.2%, a platinum crucible being used.

**Ordinary impurities** (466)—

*Test solution*: methanol.

*Standard solution*: methanol.

*Application volume*: 10  $\mu$ L.

*Eluant*: a mixture of toluene and isopropyl alcohol (90:10), in a nonequilibrated chamber.

*Visualization*: 5.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (800:700:1.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 35 mg of progesterone to a 50-mL volumetric flask, add *Mobile phase* to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Betamethasone Acetate RS in *Mobile phase*, and quantitatively dilute with *Mobile phase* to obtain a solution containing about 0.5 mg per mL. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.1 mg of USP Betamethasone Acetate RS per mL.

**Assay preparation**—Transfer about 50 mg of Betamethasone Acetate, accurately weighed, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 3 for progesterone and 1.0 for betamethasone acetate; the resolution,  $R$ , between the analyte and internal standard peaks is not less than 2; and the relative standard deviation for replicate injections is not more than 2.0%.

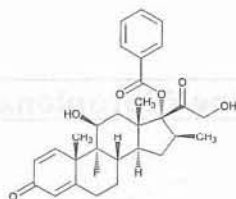
**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{24}H_{31}FO_6$  in the portion of Betamethasone Acetate taken by the formula:

$$500C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Betamethasone Acetate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Betamethasone Benzoate



$C_{29}H_{33}FO_6$  496.57  
 Pregna-1,4-diene-3,20-dione, 17-(benzoyloxy)-9-fluoro-11,21-dihydroxy-16-methyl-, (11 $\beta$ ,16 $\beta$ )-;  
 9-Fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 17-benzoate [22298-29-9].

### DEFINITION

Betamethasone Benzoate contains NLT 98.0% and NMT 102.0% of betamethasone benzoate ( $C_{29}H_{33}FO_6$ ), calculated on the dried basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197M)

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile and water (60:40)

**Internal standard solution:** 0.6 mg/mL of

betamethasone dipropionate in methanol

**Standard stock solution:** 0.6 mg/mL of USP

Betamethasone Benzoate RS in methanol

**Standard solution:** 0.2 mg/mL of USP Betamethasone

Benzoate RS prepared by mixing 5.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution*

**Sample stock solution:** 0.6 mg/mL of Betamethasone Benzoate in methanol

**Sample solution:** 0.2 mg/mL of Betamethasone Benzoate prepared by mixing 5.0 mL of *Sample stock solution* and 10.0 mL of *Internal standard solution*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4-mm  $\times$  30-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 15  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for betamethasone benzoate and betamethasone dipropionate are 1.0 and 1.4, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between betamethasone benzoate and the internal standard

**Relative standard deviation:** NMT 2.0% from three replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of betamethasone benzoate ( $C_{29}H_{33}FO_6$ ) in the portion of Betamethasone Benzoate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of the betamethasone benzoate peak to the internal standard peak from the *Sample solution*

$R_S$  = peak response ratio of the betamethasone benzoate peak to the internal standard peak from the *Standard solution*

$C_S$  = concentration of USP Betamethasone Benzoate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Betamethasone Benzoate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

#### • RELATED STEROIDS

**Standard solution A:** 5 mg/mL of USP Betamethasone Benzoate RS in methanol

**Standard solution B:** 100  $\mu$ g/mL of USP

Betamethasone Benzoate RS from *Standard solution A* in methanol

**Sample solution:** 20.0 mg/mL of Betamethasone Benzoate in methanol

**Chromatographic system**

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Toluene, acetone, and methanol (75:25:4)

**Analysis**

**Samples:** *Standard solutions* and *Sample solution*  
 Proceed as directed in the chapter. Examine the plate under short-wavelength UV light.

**Acceptance criteria:** The principal spot from the *Sample solution* corresponds in  $R_f$  value to that of *Standard solution A*; and the *Sample solution* shows NMT 3 additional spots, the intensity and size of which do not exceed those of the spot from *Standard solution B*.

### SPECIFIC TESTS

#### • OPTICAL ROTATION, *Specific Rotation* <781S>

**Sample solution:** 40 mg/mL, in dioxane

**Acceptance criteria:** +60° to +66°

#### • LOSS ON DRYING <731>

**Sample:** 200 mg

**Analysis:** Dry the *Sample* at 105° for 3 h.

**Acceptance criteria:** NMT 0.5%

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store between 2° and 30°.

• **USP REFERENCE STANDARDS <11>**  
 USP Betamethasone Benzoate RS

## Betamethasone Benzoate Gel

### DEFINITION

Betamethasone Benzoate Gel contains an amount of betamethasone benzoate ( $C_{29}H_{33}FO_6$ ) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone benzoate ( $C_{29}H_{33}FO_6$ ).

### IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Methanol, acetonitrile, and water (23:9:18)

**Internal standard solution:** 250  $\mu$ g/mL of USP

Methyltestosterone RS in methanol

**Standard stock solution:** 0.5 mg/mL of USP

Betamethasone Benzoate RS in methanol

**Standard solution:** 0.05 mg/mL of USP Betamethasone Benzoate RS prepared as follows. Transfer 5.0 mL of



*Standard stock solution* to a 50-mL volumetric flask, add 10.0 mL of *Internal standard solution*, and dilute with methanol to volume.

**Sample solution:** 0.05 mg/mL of betamethasone benzoate in methanol prepared as follows. Transfer a portion of Gel, nominally equivalent to 0.5 mg of betamethasone benzoate, into a 125-mL separatory funnel. Add 20 mL of water and 2 mL of saturated sodium acetate solution, shake to disperse the Gel, and add 2.0 mL of *Internal standard solution*. Extract this solution with one 50-mL portion of chloroform, followed by three 40-mL portions of chloroform. Discard the aqueous layer. Wash the chloroform extract with 10 mL of water, allow to stand for 10 min, then pass through chloroform-wetted glass fiber filter paper and anhydrous sodium sulfate into a suitable container. Evaporate to dryness under a vacuum at 30°. Dissolve the residue in 10 mL of methanol.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 236 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for methyltestosterone and betamethasone benzoate are about 1.0 and 1.33, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between methyltestosterone and betamethasone benzoate

**Relative standard deviation:** NMT 1.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone benzoate ( $C_{29}H_{33}FO_6$ ) in the portion of Gel taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of the betamethasone benzoate peak to the internal standard peak from the *Sample solution*

$R_S$  = peak response ratio of the betamethasone benzoate peak to the internal standard peak from the *Standard solution*

$C_S$  = concentration of USP Betamethasone Benzoate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of betamethasone benzoate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

#### ADDITIONAL REQUIREMENTS

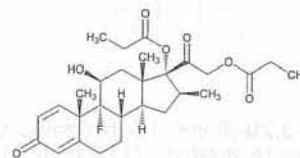
- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°. Protect from freezing.

#### • USP REFERENCE STANDARDS (11)

USP Betamethasone Benzoate RS

USP Methyltestosterone RS

## Betamethasone Dipropionate



$C_{28}H_{37}FO_7$

504.59

Pregna-1,4-diene-3,20-dione, 9-fluoro-11-hydroxy-16-methyl-17,21-bis(1-oxopropoxy)-, (11β,16β); 9-Fluoro-11β,17,21-trihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [5593-20-4].

#### DEFINITION

Betamethasone Dipropionate contains NLT 97.0% and NMT 103.0% of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**

- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Sample solution:** 1 mg/mL, in chloroform

**Chromatographic system**

**Developing solvent system:** Chloroform and acetone (7:1)

**Acceptance criteria:** Meets the requirements

#### ASSAY

- **PROCEDURE**

**Mobile phase:** Acetonitrile and water (1 in 2) such that the retention times for betamethasone dipropionate and beclomethasone dipropionate are 14 and 18 min, respectively. Degas by sonicating for 5–10 min. Do not leave *Mobile phase* in the column overnight, but flush the system after use with water for 15 min, followed by methanol for 15 min.

**Diluent:** Acetic acid and methanol (1 in 1000)

**Internal standard solution:** 0.9 mg/mL of USP

Beclomethasone Dipropionate RS in *Diluent*

**Standard stock solution:** 0.6 mg/mL of USP

Betamethasone Dipropionate RS in *Diluent*

**Standard solution:** 0.3 mg/mL of USP Betamethasone

Dipropionate RS and 0.45 mg/mL of USP

Beclomethasone Dipropionate RS, prepared by combining 5.0 mL each of the *Internal standard solution* and the *Standard stock solution*

**Sample stock solution:** 0.6 mg/mL of Betamethasone Dipropionate in *Diluent*

**Sample solution:** 0.3 mg/mL of Betamethasone Dipropionate, prepared by combining 5.0 mL each of the *Internal standard solution* and the *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 or 240 nm

**Column:** 4-mm × 30-cm; packing L1

**Injection volume:** 5–25 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Peak area ratios:** The lowest and highest peak area ratios of three successive injections agree within 2.0%.



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ) in the portion of Betamethasone Dipropionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak height ratio of betamethasone dipropionate to the internal standard from the *Sample solution*

$R_S$  = peak height ratio of betamethasone dipropionate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Betamethasone Dipropionate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Betamethasone Dipropionate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–103.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.2%, using a platinum crucible

- **ORGANIC IMPURITIES**

**Mobile phase:** Acetonitrile and water (65:35)

**System suitability solution:** 0.05 mg/mL of USP Betamethasone Dipropionate RS and 0.05 mg/mL of USP Betamethasone Valerate RS in *Mobile phase*

**Sample solution:** 0.3 mg/mL of Betamethasone Dipropionate in *Mobile phase*. Shake until dissolved.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 4.0 between betamethasone valerate and betamethasone dipropionate

**Column efficiency:** NLT 8000 theoretical plates

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Betamethasone Dipropionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses for all the peaks

**Acceptance criteria**

**Individual impurities:** NMT 1.0%

**Total impurities:** NMT 2.0%

**SPECIFIC TESTS**

- **OPTICAL ROTATION, Specific Rotation** (781S)

**Sample solution:** 10 mg/mL, in dioxane

**Acceptance criteria:** +63° to +70°

- **LOSS ON DRYING** (731)

**Analysis:** Dry at 105° for 3 h.

**Acceptance criteria:** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

- **USP REFERENCE STANDARDS** (11)

USP Beclomethasone Dipropionate RS

USP Betamethasone Dipropionate RS

USP Betamethasone Valerate RS

## Betamethasone Dipropionate Topical Aerosol

» Betamethasone Dipropionate Topical Aerosol is a solution, in suitable propellants in a pressurized container, of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in tight, pressurized containers, and avoid exposure to excessive heat. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—

USP Beclomethasone Dipropionate RS

USP Betamethasone Dipropionate RS

**Thin-layer chromatographic identification test** (201)—

**Test solution**—Place the container in a dry ice-methanol bath for about 5 minutes. Open the can by means of a tube-cutter, and allow the propellant to evaporate under a gentle stream of nitrogen for about 1 hour. Transfer about 3 mL of the residue to a 50-mL centrifuge tube. Add 10 mL of a mixture of methanol and water (4:1), and shake vigorously. Centrifuge to clarify.

**Standard solution:** USP Betamethasone Dipropionate RS in methanol containing 3.2 mg per mL.

**Application volume:** 25  $\mu$ L.

**Developing solvent system:** a mixture of toluene and ethyl acetate (1:1).

**Procedure**—Proceed as directed in the chapter. Spray the plate with a mixture of sulfuric acid, methanol, and nitric acid (10:10:1), and heat at 105° for 15 minutes.

**Change to read:**

**Other requirements**—It meets the requirements for *Pressure Test*, *Minimum Fill*, and *Leakage Test* under **Topical Aerosols** (603) (CN 1-May-2017).

**Assay**

**Mobile phase**—Prepare as directed for *Mobile phase* in the *Assay* under *Betamethasone Dipropionate*.

**Internal standard solution**—Prepare a solution of USP Beclomethasone Dipropionate RS, having a known concentration of about 0.90 mg per mL, in isopropyl alcohol containing glacial acetic acid (1 in 1000).

**Standard preparation**—Prepare a solution of USP Betamethasone Dipropionate RS, having a known concentration of about 0.642 mg per mL, in isopropyl alcohol containing acetic acid (1 in 1000). Transfer 10.0 mL of this solution and 10.0 mL of *Internal standard solution* to a 100-mL volumetric flask, add isopropyl alcohol containing acetic acid (1 in 1000) to volume, and mix, to obtain a solution having known concentrations of about 0.09 mg of beclomethasone dipropionate and about 0.0642 mg of betamethasone dipropionate per mL.

**Assay preparation**—Discharge the entire contents of the container of Topical Aerosol into a 100-mL volumetric flask. Allow the solution to warm to room temperature slowly to prevent it from boiling out of the flask, then evaporate the propellant by swirling the flask in a water bath at about 25°



until the solution stops bubbling. Add 10.0 mL of *Internal standard solution*, and dilute with glacial acetic acid in isopropyl alcohol (1 in 1000) to volume. Pass the solution through a 0.45- $\mu$ m filter.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Betamethasone Dipropionate*. Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_3$ ) equivalent to the quantity of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ) in the container of the Topical Aerosol taken by the formula:

$$(392.46/504.60)(100C)(R_U / R_S)$$

in which 392.46 and 504.60 are the molecular weights of betamethasone and betamethasone dipropionate, respectively; C is the concentration, in mg per mL, of USP Betamethasone Dipropionate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak height ratios of the betamethasone dipropionate and beclomethasone dipropionate peaks in the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Dipropionate Cream

### DEFINITION

Betamethasone Dipropionate Cream contains an amount of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ( $C_{22}H_{29}FO_3$ ), in a suitable cream base.

### IDENTIFICATION

#### A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Standard solution:** 150  $\mu$ g/mL of USP Betamethasone Dipropionate RS in chloroform

**Sample solution:** Nominally equivalent to 150  $\mu$ g/mL of betamethasone dipropionate, prepared as follows. Transfer 1.5 g of Cream to a glass-stoppered, 50-mL centrifuge tube. Add 15 mL of a methanol-hydrochloric acid solution prepared by mixing 1 volume of dilute hydrochloric acid (1 in 120) with 4 volumes of methanol. Shake to obtain a homogeneous mixture. Add 30 mL of solvent hexane, mix for 10 min, and centrifuge. Using a syringe, transfer the lower aqueous phase to a second centrifuge tube, and add 20 mL of water. Extract this aqueous mixture with chloroform by shaking, centrifuging, and removing the lower, chloroform phase with a syringe. Evaporate the chloroform on a steam bath with the aid of a stream of nitrogen to dryness, cool, and dissolve the residue in chloroform.

#### Chromatographic system

**Application volume:** 40  $\mu$ L

**Developing solvent system:** Chloroform and acetone (7:1)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Proceed as directed in the chapter.

**Acceptance criteria:** Meets the requirements

### ASSAY

#### PROCEDURE

**Mobile phase:** Acetonitrile and water (1 in 2) such that the retention times for betamethasone dipropionate and beclomethasone dipropionate are 14 and 18 min, respectively. Degas by sonicating for 5–10 min. Do not leave the *Mobile phase* in the column overnight, but flush the system after use with water for 15 min, followed by methanol for 15 min.

**Diluent:** Acetic acid in methanol (1 in 1000)

**Internal standard solution:** 0.45 mg/mL of USP

Beclomethasone Dipropionate RS in *Diluent*

**Standard stock solution:** 0.2 mg/mL of USP Betamethasone Dipropionate RS in *Diluent*

**Standard solution:** 0.133 mg/mL of USP Betamethasone Dipropionate RS and 0.15 mg/mL of USP Beclomethasone Dipropionate RS prepared by combining 10.0 mL of the *Standard stock solution* and 5.0 mL of the *Internal standard solution*

**Sample solution:** Nominally equivalent to 0.1 mg/mL of betamethasone, prepared as follows. Transfer a portion of Cream, equivalent to 2 mg of betamethasone dipropionate (1.6 mg of betamethasone), into a capped 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution* and 10.0 mL of *Diluent*. Heat in a water bath at 60°, shaking intermittently, until the Cream melts. Remove from the bath, and shake vigorously until the Cream has resolidified. Repeat the heating and shaking. Freeze in an ice-methanol bath for 15 min, and centrifuge at 2500 rpm for 5 min. Use the supernatant.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 or 240 nm

**Column:** 4-mm  $\times$  30-cm; packing L1

**Injection volume:** 5–25  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Peak area ratios:** The lowest and highest peak area ratios of three successive injections agree within 2.0%.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone ( $C_{22}H_{29}FO_3$ ) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak height ratio of betamethasone dipropionate to the internal standard from the *Sample solution*

$R_S$  = peak height ratio of betamethasone dipropionate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Betamethasone Dipropionate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of betamethasone, 392.46

$M_{r2}$  = molecular weight of betamethasone dipropionate, 504.59

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

• **MINIMUM FILL (755):** Meets the requirements

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers. Store at 25°, excursions permitted between 15° and 30°. Protect from freezing.

• **USP REFERENCE STANDARDS (11)**

USP Beclomethasone Dipropionate RS

USP Betamethasone Dipropionate RS

## Betamethasone Dipropionate Lotion

### DEFINITION

Betamethasone Dipropionate Lotion contains an amount of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ( $C_{22}H_{29}FO_3$ ), in a suitable lotion base.



**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 150 µg/mL of USP Betamethasone Dipropionate RS in chloroform

**Sample solution:** Nominally 150 µg/mL of betamethasone dipropionate, prepared as follows. Transfer a portion of Lotion, equivalent to 0.6 mg of betamethasone dipropionate, to a 50-mL vial; add 10 mL of 0.1 N hydrochloric acid; then add 4 mL of chloroform. Disperse on a vortex mixer for about 1 min, shake vigorously for 10 min, and centrifuge at 2000 rpm for about 5 min. Transfer the chloroform layer to a suitable vial.

**Chromatographic system**

**Application volume:** 40 µL

**Developing solvent system:** Chloroform and acetone (7:1)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in the chapter.

**Acceptance criteria:** Meets the requirements

**ASSAY**

- **PROCEDURE**

**Mobile phase:** Acetonitrile and water (1 in 2) such that the retention times for betamethasone dipropionate and beclomethasone dipropionate are 14 and 18 min, respectively. Degas by sonicating for 5–10 min. Do not leave the *Mobile phase* in the column overnight, but flush the system after use with water for 15 min, followed by methanol for 15 min.

**Internal standard solution:** 0.9 mg/mL of USP Beclomethasone Dipropionate RS in chloroform

**Standard stock solution A:** 0.6 mg/mL of USP Betamethasone Dipropionate RS in chloroform

**Standard stock solution B:** 0.3 mg/mL of betamethasone dipropionate and 0.45 mg/mL of beclomethasone dipropionate, prepared by combining 5.0 mL each of *Internal standard solution* and *Standard stock solution A*

**Standard solution:** To 10.0 mL of 0.1 N hydrochloric acid in a capped 5-mL centrifuge tube, add 4.0 mL of *Standard stock solution B*. Cap the tube, and shake vigorously for about 2 min, or disperse on a vortex mixer for about 1 min. Centrifuge at 2500 rpm for about 3 min. Transfer the chloroform phase to a suitable vial. Evaporate the chloroform under a stream of nitrogen at a slightly elevated temperature to dryness. Cool the vial to room temperature, add 4.0 mL of methanol, and swirl to dissolve the residue.

**Sample solution:** Nominally 0.23 mg/mL of betamethasone, prepared as follows. Transfer a portion of Lotion, equivalent to 1.2 mg of betamethasone dipropionate (0.93 mg of betamethasone), into a capped 50-mL centrifuge tube. Add 10.0 mL of 0.1 N hydrochloric acid, shake to disperse, then add 2.0 mL of *Internal standard solution* and 2.0 mL of chloroform. Cap, and shake vigorously for about 2 min, or disperse on a vortex mixer for about 1 min. Centrifuge at 2500 rpm for about 3 min. Transfer the chloroform phase to a suitable vial. Evaporate the chloroform under a stream of nitrogen at a slightly elevated temperature to dryness. Cool the vial to room temperature, add 4.0 mL of methanol, and swirl to dissolve the residue.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 or 240 nm

**Column:** 4-mm × 30-cm; packing L1

**Injection volume:** 5–25 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Peak area ratios:** The lowest and highest peak area ratios of three successive injections agree within 2.0%.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak height ratio of betamethasone dipropionate to the internal standard from the *Sample solution*

$R_S$  = peak height ratio of betamethasone dipropionate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Betamethasone Dipropionate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of betamethasone, 392.46

$M_{r2}$  = molecular weight of betamethasone dipropionate, 504.59

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- **MINIMUM FILL (755):** Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°. Protect from light and freezing.
- **USP REFERENCE STANDARDS (11)**  
USP Beclomethasone Dipropionate RS  
USP Betamethasone Dipropionate RS

**Betamethasone Dipropionate Ointment****DEFINITION**

Betamethasone Dipropionate Ointment contains an amount of betamethasone dipropionate ( $C_{28}H_{37}FO_7$ ) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ), in a suitable ointment base.

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 150 µg/mL of USP Betamethasone Dipropionate RS in chloroform

**Sample solution:** Nominally 150 µg/mL of betamethasone dipropionate, prepared as follows. Transfer 1.5 g of Ointment to a glass-stoppered, 50-mL centrifuge tube. Add 15 mL of methanol–hydrochloric acid solution prepared by mixing 1 volume of dilute hydrochloric acid (1 in 120) with 4 volumes of methanol. Shake to obtain a homogeneous mixture. Add 30 mL of solvent hexane, mix for 10 min, and centrifuge. Using a syringe, transfer the lower aqueous phase to a second centrifuge tube, and add 20 mL of water. Extract this aqueous mixture with chloroform by shaking, centrifuging, and removing the lower, chloroform



phase with a syringe. Evaporate the chloroform on a steam bath with the aid of a stream of nitrogen to dryness, cool, and dissolve the residue in chloroform.

#### Chromatographic system

Application volume: 40  $\mu$ L

Developing solvent system: Chloroform and acetone (7:1)

#### Analysis

Samples: *Standard solution* and *Sample solution*  
Proceed as directed in the chapter.

Acceptance criteria: Meets the requirements

### ASSAY

#### PROCEDURE

**Mobile phase:** Acetonitrile and water (1 in 2) such that the retention times for betamethasone dipropionate and beclomethasone dipropionate are 14 and 18 min, respectively. Degas by sonicating for 5–10 min. Do not leave the *Mobile phase* in the column overnight, but flush the system after use with water for 15 min, followed by methanol for 15 min.

**Diluent:** Acetic acid and alcohol (1 in 1000)

**Internal standard solution:** 0.45 mg/mL of USP

Beclomethasone Dipropionate RS in *Diluent*

**Standard stock solution:** 0.2 mg/mL of USP

Betamethasone Dipropionate RS in *Diluent*

**Standard solution:** 0.133 mg/mL of USP

Betamethasone Dipropionate RS and 0.15 mg/mL of USP Beclomethasone Dipropionate RS prepared by combining 10.0 mL of the *Standard stock solution* and 5.0 mL of the *Internal standard solution*

**Sample solution:** Nominally equivalent to 0.1 mg/mL of betamethasone, prepared as follows. Transfer a portion of Ointment, equivalent to 2 mg of betamethasone dipropionate (1.6 mg of betamethasone), into a capped 50-mL centrifuge tube. Add 5.0 mL of *Internal standard solution* and 10.0 mL of *Diluent*. Heat in a water bath at 70°, shaking intermittently, until the sample melts. Remove from the bath, and shake vigorously until the Ointment has solidified. Repeat the heating and shaking. Freeze in an ice-methanol bath for 15 min, and centrifuge at 2500 rpm for 5 min. Use the supernatant.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 or 240 nm

**Column:** 4-mm  $\times$  30-cm; packing L1

**Injection volume:** 5–25  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Peak area ratios:** The lowest and highest peak area ratios of three successive injections agree within 2.0%.

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak height ratio of betamethasone dipropionate to the internal standard from the *Sample solution*

$R_S$  = peak height ratio of betamethasone dipropionate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Betamethasone Dipropionate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of betamethasone from the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of betamethasone, 392.46

$M_{r2}$  = molecular weight of betamethasone dipropionate, 504.59

Acceptance criteria: 90.0%–110.0%

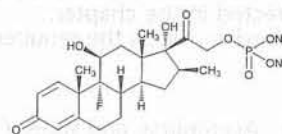
### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers. Store at 25°, with excursions permitted between 15° and 30°. Protect from freezing.
- **USP REFERENCE STANDARDS (11)**  
USP Beclomethasone Dipropionate RS  
USP Betamethasone Dipropionate RS

## Betamethasone Sodium Phosphate



$C_{22}H_{28}FN_2O_8P$

516.40

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17-dihydroxy-16-methyl-21-(phosphonooxy)-, disodium salt, (11 $\beta$ ,16 $\beta$ ); 9-Fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 21-(disodium phosphate) [151-73-5].

### DEFINITION

Betamethasone Sodium Phosphate contains NLT 97.0% and NMT 103.0% of betamethasone sodium phosphate ( $C_{22}H_{28}FN_2O_8P$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 1 mg/mL of USP Betamethasone Sodium Phosphate RS in methanol

**Sample solution:** 1 mg/mL of Betamethasone Sodium Phosphate in methanol

#### Chromatographic system

Application volume: 10  $\mu$ L

**Developing solvent system:** 500 mL of butyl alcohol and 200 mL of dilute hydrochloric acid (1 in 12). Place in a separatory funnel, and mix. Use the organic layer as the developing solvent.

**Spray reagent:** Sulfuric acid, methanol, and nitric acid (10:10:1)

#### Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter, except to spray the plate with *Spray reagent*, and heat at 105° for 10 min.

Acceptance criteria: Meets the requirements

- **C. IDENTIFICATION TESTS—GENERAL, Sodium (191) and Phosphate (191)**

**Analysis:** Ignite it at 800° (see *Residue on Ignition* (281)).

Acceptance criteria: The residue meets the requirements for sodium and phosphate.

### ASSAY

#### PROCEDURE

**Mobile phase:** Methanol and 0.07 M anhydrous monobasic potassium phosphate (3:2)

**Diluent:** Methanol and water (3:2)

**Standard solution:** 0.17 mg/mL of USP Betamethasone Sodium Phosphate RS in *Diluent*



**Sample solution:** 0.17 mg/mL of Betamethasone Sodium Phosphate in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2

**Relative standard deviation:** NMT 3.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of betamethasone sodium phosphate ( $C_{22}H_{28}FNa_2O_8P$ ) in the portion of Betamethasone Sodium Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Betamethasone Sodium Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Betamethasone Sodium Phosphate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–103.0% on the anhydrous basis

**IMPURITIES**

• **LIMIT OF PHOSPHATE IONS**

**Standard phosphate solution and Phosphate reagent**

**A:** Prepare as directed in *Phosphate in Reagents* (see *Reagents, Indicators, and Solutions—General Tests for Reagents*).

**Phosphate reagent B:** Dissolve 350 mg of *p*-methylaminophenol sulfate in 50 mL of water. Add 20 g of sodium metabisulfite, mix to dissolve, and dilute with water to 100 mL.

**Standard solution:** Dilute 5.0 mL of *Standard phosphate solution* in a mixture of 10 mL of water and 5 mL of 2 N sulfuric acid contained in a 25-mL volumetric flask, by warming if necessary. Add 1 mL each of *Phosphate reagent A* and *Phosphate reagent B*, dilute with water to 25.0 mL, mix, and allow to stand at room temperature for 30 min.

**Sample solution:** Dissolve 50 mg of Betamethasone Sodium Phosphate in a mixture of 10 mL of water and 5 mL of 2 N sulfuric acid contained in a 25-mL volumetric flask, by warming if necessary. Add 1 mL each of *Phosphate reagent A* and *Phosphate reagent B*, dilute with water to 25.0 mL, mix, and allow to stand at room temperature for 30 min.

**Instrumental conditions**

**Mode:** Vis

**Analytical wavelength:** Maximum absorbance at about 730 nm

**Cell:** 1 cm

**Blank:** Water

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** The absorbance of the *Sample solution* is NMT that of the *Standard solution*. The limit is 1.0% of phosphate ( $PO_4$ ).

• **LIMIT OF FREE BETAMETHASONE**

**Sample stock solution:** 1.0 mg/mL of Betamethasone Sodium Phosphate in water, prepared as follows. Dissolve 25.0 mg of Betamethasone Sodium Phosphate in water to make 25.0 mL.

**Sample solution:** Transfer 5.0 mL of the *Sample stock solution* to a separator, and extract with three 25-mL portions of chloroform. Filter each extract through a

chloroform-saturated cotton pledget, combining the filtrates in a conical flask. Evaporate the chloroform on a steam bath to dryness with the aid of a current of air, and dissolve the residue in methanol to make 25.0 mL.

**Blank solution:** Transfer 5.0 mL of water to a separator. Proceed as directed in *Sample solution*, beginning with "extract with three 25-mL portions of chloroform".

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** Maximum absorbance at about 239 nm

**Cell:** 1 cm

**Blank:** *Blank solution*

**Analysis**

**Sample:** *Sample solution*

Calculate the quantity, in mg, of free betamethasone in the portion of Betamethasone Sodium Phosphate taken:

$$\text{Result} = A \times 3.125$$

$A$  = absorbance of the *Sample solution*

**Acceptance criteria:** NMT 0.25 mg (1.0%)

**SPECIFIC TESTS**

• **OPTICAL ROTATION, Specific Rotation (781S)**

**Sample solution:** 10 mg/mL

**Acceptance criteria:** +99° to +105°

• **WATER DETERMINATION, Method I (921):** NMT 10.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Betamethasone Sodium Phosphate RS

## Betamethasone Sodium Phosphate Injection

» Betamethasone Sodium Phosphate Injection is a sterile solution of Betamethasone Sodium Phosphate in Water for Injection. It contains an amount of betamethasone sodium phosphate ( $C_{22}H_{28}FNa_2O_8P$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage—**Preserve in single-dose or in multiple-dose containers, preferably of Type I glass.

**USP Reference standards (11)—**

USP Betamethasone Sodium Phosphate RS

USP Endotoxin RS

**Identification—**Dilute the Injection with methanol, if necessary, to obtain a solution containing about 2 mg of betamethasone sodium phosphate per mL. Separately apply 10 µL of this test solution and 10 µL of a solution of USP Betamethasone Sodium Phosphate RS in methanol containing 2 mg per mL to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with chromatographic silica gel mixture. Develop the chromatogram in an equilibrated chamber containing *n*-butyl alcohol previously shaken with 1 N hydrochloric acid, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, air-dry, then spray with a mixture of sulfuric acid, methanol, and nitric acid (10:10:1). Heat the plate at 105° for 10 minutes; the  $R_f$  value of the principal spot from the test solution corresponds to that obtained from the Standard solution.



**Bacterial Endotoxins Test** (85)—It contains not more than 29.2 USP Endotoxin Units per mg of betamethasone.

**pH** (791): between 8.0 and 9.0.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and 0.05 M monobasic potassium phosphate (1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 100 mg of butylparaben to a 100-mL volumetric flask, add methanol to volume, and mix.

**Standard preparation**—Using an accurately weighed quantity of USP Betamethasone Sodium Phosphate RS, prepare a solution in water containing 4 mg per mL. Transfer 3.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with water to volume, and mix to obtain a solution having a known concentration of about 0.5 mg of USP Betamethasone Sodium Phosphate RS per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 9 mg of betamethasone, to a 25-mL volumetric flask. Add 5.0 mL of the *Internal standard solution*, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the analyte and internal standard peaks is not less than 5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 2.4 for butylparaben and 1.0 for betamethasone sodium phosphate. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in each mL of the Injection taken by the formula:

$$(392.47 / 516.41)(25C / V)(R_U / R_S)$$

in which 392.47 and 516.41 are the molecular weights of betamethasone and betamethasone sodium phosphate, respectively; *C* is the concentration, in mg per mL, of USP Betamethasone Sodium Phosphate RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Sodium Phosphate and Betamethasone Acetate Injectable Suspension

» Betamethasone Sodium Phosphate and Betamethasone Acetate Injectable Suspension is a sterile preparation of Betamethasone Sodium Phosphate in solution and Betamethasone Acetate in suspension in Water for Injection. It contains an amount of betamethasone sodium phosphate ( $C_{22}H_{28}FNa_2O_8P$ ) equivalent to not less

than 90.0 percent and not more than 115.0 percent of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ), and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of betamethasone acetate ( $C_{24}H_{31}FO_6$ ).

**Packaging and storage**—Preserve in multiple-dose containers, preferably of Type I glass.

#### USP Reference standards (11)—

USP Betamethasone Acetate RS

USP Betamethasone Sodium Phosphate RS

USP Endotoxin RS

#### Identification—

**A: Thin-layer chromatographic identification test** (201)—

**Test solution**—Dilute 2 mL with 2 mL of methanol.

**Standard solution**—Prepare a solution of USP

Betamethasone Sodium Phosphate RS in a mixture of methanol and water (1:1) having a concentration of 2 mg per mL.

**Developing solvent system, Spray reagent, and Procedure**—Proceed as directed for *Identification test B* under *Betamethasone sodium phosphate*.

**B: Test solution**—Use the *Test solution* prepared for *Identification test A*.

**Standard solution**—Prepare a solution of USP Betamethasone Acetate RS in a mixture of methanol and water (1:1) having a concentration of 1.5 mg per mL.

**Developing solvent system and Procedure**—Proceed as directed for *Identification test B* under *Betamethasone*.

**Bacterial Endotoxins Test** (85)—It contains not more than 29.2 USP Endotoxin Units per mg of betamethasone.

**pH** (791): between 6.8 and 7.2.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and 0.075 M monobasic potassium phosphate (7:5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 50 mg of methyltestosterone to a 50-mL volumetric flask, add methanol to volume, and mix.

**Standard preparation**—Transfer about 63 mg of USP Betamethasone Sodium Phosphate RS, accurately weighed, to a 25-mL volumetric flask, add *Mobile phase* to volume, and mix (*Solution 1*). Transfer about 45 mg of USP Betamethasone Acetate RS, accurately weighed, to a 25-mL volumetric flask, add methanol to volume, and mix (*Solution 2*). Pipet 5 mL each of *Solution 1* and *Solution 2* into a 100-mL volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having known concentrations of about 126 µg of USP Betamethasone Sodium Phosphate RS per mL and 90 µg of USP Betamethasone Acetate RS per mL.

**Assay preparation**—Using a “To contain” pipet transfer an accurately measured volume of the well-mixed Injectable Suspension, equivalent to about 9 mg of betamethasone acetate, to a 100-mL volumetric flask. Rinse the pipet with about 10 mL of *Mobile phase*, collecting the rinse in the volumetric flask. Add 10.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the betamethasone phosphate and betamethasone acetate peaks is not less than 5.0, and the resolution, *R*, between



the betamethasone acetate and internal standard peaks is not less than 3.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for betamethasone phosphate, 1.7 for methyltestosterone, and 1.0 for betamethasone acetate. Calculate the quantity, in mg, of betamethasone acetate ( $C_{24}H_{31}FO_6$ ) in each mL of the Injectable Suspension taken by the formula:

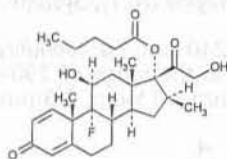
$$0.1C / V(R_U / R_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Betamethasone Acetate RS in the *Standard preparation*; V is the volume, in mL, of Injectable Suspension taken; and  $R_U$  and  $R_S$  are the peak response ratios obtained for betamethasone acetate and methyltestosterone from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_5$ ) equivalent to the quantity of betamethasone sodium phosphate ( $C_{22}H_{28}FNa_2O_8P$ ), in each mL of the Injectable Suspension taken by the formula:

$$(392.46/516.41)(0.1C/V)(R_U / R_S)$$

in which 392.46 and 516.41 are the molecular weights of betamethasone and betamethasone sodium phosphate, respectively; C is the concentration, in  $\mu$ g per mL, of USP Betamethasone Sodium Phosphate RS in the *Standard preparation*; V is the volume, in mL, of Injectable Suspension taken; and  $R_U$  and  $R_S$  are the peak response ratios obtained for betamethasone phosphate and methyltestosterone from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Valerate



$C_{27}H_{37}FO_6$  476.58

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16-methyl-17-[(1-oxopentyl)oxy]-, (11 $\beta$ ,16 $\beta$ )-

9-Fluoro-11 $\beta$ ,17,21-trihydroxy-16 $\beta$ -methylpregna-1,4-diene-3,20-dione 17-valerate [2152-44-5].

» Betamethasone Valerate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{27}H_{37}FO_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Beclomethasone Dipropionate RS

USP Betamethasone Valerate RS

**Identification**—

A: Infrared Absorption (197M).

B: Thin-Layer Chromatographic Identification Test (201)—

Test solution: 1 mg per mL, in alcohol.

Developing solvent system: a mixture of toluene and ethyl acetate (1:1).

**Procedure**—Proceed as directed in the chapter. Spray the plate with a mixture of sulfuric acid, methanol, and nitric acid (10:10:1), and heat at 105° for 15 minutes.

**Specific rotation** (781S): between +75° and +82°.

Test solution: 10 mg per mL, in dioxane.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.2%, a platinum crucible being used.

**Chromatographic purity**—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, water, and glacial acetic acid (550:450:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Test solution**—Transfer about 4 mg of Betamethasone Valerate, accurately weighed, to a suitable flask. Add 10 mL of *Mobile phase*, and shake until dissolved.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between betamethasone valerate and any impurity is not less than 1.5; and the column efficiency is not less than 9000 theoretical plates.

**Procedure**—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Betamethasone Valerate taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of all the peak responses: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and water (3:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 40 mg of beclomethasone dipropionate to a 100-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix.

**Standard preparation**—Transfer about 30 mg of USP Betamethasone Valerate RS, accurately weighed, to a 50-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of *Internal standard solution*, and mix to obtain a solution having a known concentration of about 0.2 mg of USP Betamethasone Valerate RS per mL.

**Assay preparation**—Transfer about 60 mg of Betamethasone Valerate, accurately weighed, to a 100-mL volumetric flask, add a solution of glacial acetic acid in methanol (1 in 1000) to volume, and mix. Transfer 5.0 mL of this solution to a suitable stoppered vial, add 10.0 mL of *Internal standard solution*, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.7 for beclomethasone dipropionate and 1.0 for betamethasone valerate; the resolution,  $R$ , between betamethasone valerate and beclomethasone dipropionate is not less than 4.5; and the relative standard deviation for replicate injections is not more than 2.0%.



**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{27}H_{37}FO_6$  in the portion of Betamethasone Valerate taken by the formula:

$$300C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Valerate Cream

### DEFINITION

Betamethasone Valerate Cream contains an amount of betamethasone valerate ( $C_{27}H_{37}FO_6$ ) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ( $C_{22}H_{29}FO_3$ ), in a suitable cream base.

### IDENTIFICATION

**Delete the following:**

#### ▲ A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Standard solution:** 1 mg/mL of USP Betamethasone Valerate RS in alcohol

**Sample solution:** Transfer an amount of Cream, equivalent to 2 mg of betamethasone, to a separator, add 20 mL of water and 2 mL of dilute hydrochloric acid (1 in 120), and mix. Extract with four 50-mL portions of chloroform, and combine the extracts. Filter through a cotton pledget, previously layered over with anhydrous sodium sulfate. Evaporate the filtrates on a steam bath under a stream of dry nitrogen to dryness. Dissolve the residue in alcohol to obtain a solution containing about 1 mg/mL.

**Developing solvent system:** Toluene and ethyl acetate (1:1)

**Application volume:** 10 µL

#### Analysis

**Samples:** *Sample solution* and *Standard solution*  
Proceed as directed in the chapter. Spray the plate with a mixture of sulfuric acid, methanol, and nitric acid (10:10:1), and heat at 105° for 15 min.▲USP40

**Add the following:**

▲ A. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲USP40

**Add the following:**

▲ B. The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.▲USP40

### ASSAY

**Change to read:**

#### • PROCEDURE

▲ **Solution A:** Water

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0.0	63	37
7.0	63	37
15.0	30	70
19.0	30	70
19.1	10	90
21.0	10	90
21.1	63	37
25.0	63	37

**Diluent A:** Tetrahydrofuran and water (50:50)

**Diluent B:** Acetonitrile and water (40:60)

**System suitability solution:** 25 µg/mL of USP Betamethasone Valerate RS and 10 µg/mL of USP Betamethasone Valerate Related Compound A RS in *Diluent B*. Sonicate to dissolve if necessary.

**Standard solution:** 25 µg/mL of USP Betamethasone Valerate RS in *Diluent B*. Sonicate to dissolve if necessary.

**Sample solution:** Nominally 20 µg/mL of betamethasone, prepared as follows. Transfer 1.0 mg of betamethasone from a portion of Cream to a suitable glass centrifuge tube. Add 15.0 mL of *Diluent A* and mix with a vortex mixer to disperse the sample thoroughly. Add 35.0 mL of *Diluent B* and sonicate for 10 min with intermittent shaking. Centrifuge to obtain a clear supernatant. Pass through a suitable filter of 0.2-µm pore size using a glass syringe. Discard the first 1 mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

**Column:** 4.6-mm × 15-cm; 3.5-µm packing L1

#### Temperatures

**Autosampler:** 4°

**Column:** Ambient

**Flow rate:** 1 mL/min

**Injection volume:** 100 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of betamethasone ( $C_{22}H_{29}FO_3$ ) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*



- $C_s$  = concentration of USP Betamethasone Valerate RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_u$  = nominal concentration of betamethasone in the *Sample solution* ( $\mu\text{g/mL}$ )  
 $M_{r1}$  = molecular weight of betamethasone, 392.46  
 $M_{r2}$  = molecular weight of betamethasone valerate, 476.58<sup>▲USP40</sup>

Acceptance criteria: 90.0%–110.0%

## IMPURITIES

### Add the following:

#### ▲ ORGANIC IMPURITIES

**Solution A, Solution B, Mobile phase, Diluent A, Diluent B, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution:** 0.25  $\mu\text{g/mL}$  each of USP Betamethasone RS, USP Betamethasone Valerate RS, and USP Betamethasone Valerate Related Compound A RS in *Diluent B*. Sonicate to dissolve if necessary.

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*

**Relative standard deviation:** NMT 5%, *Standard solution*

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

Calculate the percentage of each specified degradation product in the portion of Cream taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of each specified degradation product from the *Sample solution*  
 $r_s$  = peak response of the corresponding USP Reference Standard from the *Standard solution*  
 $C_s$  = concentration of the corresponding USP Reference Standard in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_u$  = nominal concentration of betamethasone in the *Sample solution* ( $\mu\text{g/mL}$ )

Calculate the percentage of each unspecified degradation product in the portion of Cream taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

- $r_u$  = peak response of each unspecified degradation product from the *Sample solution*  
 $r_s$  = peak response of betamethasone valerate from the *Standard solution*  
 $C_s$  = concentration of USP Betamethasone Valerate RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_u$  = nominal concentration of betamethasone in the *Sample solution* ( $\mu\text{g/mL}$ )  
 $M_{r1}$  = molecular weight of betamethasone, 392.46  
 $M_{r2}$  = molecular weight of betamethasone valerate, 476.58

Acceptance criteria: See Table 2. Disregard any impurity peak less than 0.1%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone	0.30	1.0
Betamethasone valerate	1.00	—
Betamethasone valerate related compound A	1.04	1.0
Any individual unspecified degradation product	—	1.0
Total degradation products	—	2.0

▲USP40

## SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- **MINIMUM FILL** (755): Meets the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers.

### Change to read:

#### • USP REFERENCE STANDARDS (11)

▲USP Betamethasone RS<sup>▲USP40</sup>

USP Betamethasone Valerate RS

▲USP Betamethasone Valerate Related Compound A RS  
 9-Fluoro-11 $\beta$ ,17-dihydroxy-16 $\beta$ -methyl-3,20-dioxopregna-1,4-dien-21-yl valerate.

$\text{C}_{27}\text{H}_{37}\text{FO}_6$  476.58<sup>▲USP40</sup>

## Betamethasone Valerate Lotion

» Betamethasone Valerate Lotion contains an amount of Betamethasone Valerate ( $\text{C}_{27}\text{H}_{37}\text{FO}_6$ ) equivalent to not less than 95.0 percent and not more than 115.0 percent of the labeled amount of betamethasone ( $\text{C}_{22}\text{H}_{29}\text{FO}_5$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Betamethasone Valerate RS

**Identification**—Mix an amount of Lotion, equivalent to about 5 mg of betamethasone, with a mixture of methanol and chloroform (2:1) to make 10 mL. Apply 20  $\mu\text{L}$  of this solution and 20  $\mu\text{L}$  of a Standard solution of USP Betamethasone Valerate RS in a mixture of methanol and chloroform (2:1) containing 0.6 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and ethyl acetate (1:1), until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. View the spots under UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.



**Minimum fill (755):** meets the requirements.

**pH (791):** between 4.0 and 6.0.

#### Assay—

**Mobile phase and Chromatographic system**—Proceed as directed in the Assay under *Betamethasone Valerate*.

**Internal standard solution**—Transfer about 50 mg of beclomethasone dipropionate to a 25-mL volumetric flask, add chloroform to volume, and mix.

**Standard preparation**—Transfer about 40 mg of USP Betamethasone Valerate RS, accurately weighed, to a 25-mL volumetric flask, add chloroform to volume, and mix. Pipet 2 mL of this solution into a 50-mL centrifuge tube, add 10 mL of 0.1 N hydrochloric acid, then add 2.0 mL of *Internal standard solution*. Insert the stopper into the tube, shake vigorously for about 2 minutes, and centrifuge to separate the phases. Using a syringe, transfer the lower, chloroform phase to a small stoppered vial. Evaporate the chloroform on a steam bath, at low heat, with the aid of a stream of nitrogen. Add 4.0 mL of a 1 in 1000 solution of glacial acetic acid in methanol, and swirl to dissolve the residue.

**Assay preparation**—Transfer an accurately weighed portion of Lotion, equivalent to about 2.5 mg of betamethasone, to a stoppered, 50-mL centrifuge tube. Add 10.0 mL of 0.1 N hydrochloric acid, insert the stopper, and shake to disperse the specimen. Add 2.0 mL of chloroform and 2.0 mL of *Internal standard solution*, insert the stopper, and proceed as directed for *Standard preparation*, beginning with "shake vigorously for about 2 minutes."

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Betamethasone Valerate*. Calculate the quantity, in mg, of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Lotion taken by the formula:

$$(392.46 / 476.59)(4C)(R_U / R_S)$$

in which 392.46 and 476.59 are the molecular weights of betamethasone and betamethasone valerate, respectively; C is the concentration, in mg per mL, of USP Betamethasone Valerate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Betamethasone Valerate Ointment

### DEFINITION

Betamethasone Valerate Ointment contains an amount of betamethasone valerate ( $C_{27}H_{37}FO_6$ ) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ), in a suitable ointment base.

### IDENTIFICATION

**Delete the following:**

#### ▲ A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Standard solution:** 1 mg/mL of USP Betamethasone Valerate RS in alcohol

**Sample solution:** Transfer the equivalent to 2 mg of betamethasone from the Ointment to a separator. Add 20 mL of water and 2 mL of dilute hydrochloric acid (1 in 120). Extract with four 50-mL portions of chloroform, and combine the extracts. Filter through a cotton pledget, previously layered over with anhydrous sodium sulfate. Evaporate the filtrates on a steam bath under a stream of dry nitrogen to dryness. Dissolve the residue in alcohol to obtain a solution containing 1 mg/mL.

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Toluene and ethyl acetate (1:1)

**Spray reagent:** A mixture of sulfuric acid, methanol, and nitric acid (10:10:1)

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

When the solvent front has moved three-fourths of the length of the plate, remove the plate from the chamber, mark the solvent front, and allow to air-dry. Locate the spots on the plate by spraying lightly with *Spray reagent*, and dry at 105° for 15 min.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. ▲USP40

#### Add the following:

- ▲ A. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ▲USP40

#### Add the following:

- ▲ B. The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay. ▲USP40

### ASSAY

#### Change to read:

#### • PROCEDURE

▲Solution A: Water

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	63	37
7.0	63	37
15.0	30	70
19.0	30	70
19.1	10	90
21.0	10	90
21.1	63	37
25.0	63	37

Diluent A: Tetrahydrofuran and water (50:50)

Diluent B: Acetonitrile and water (40:60)

System suitability solution: 25  $\mu$ g/mL of USP

Betamethasone Valerate RS and 10  $\mu$ g/mL of USP Betamethasone Valerate Related Compound A RS in *Diluent B*. Sonicate to dissolve if necessary.

**Standard solution:** 25  $\mu$ g/mL of USP Betamethasone Valerate RS in *Diluent B*. Sonicate to dissolve if necessary.

**Sample solution:** Nominally 20  $\mu$ g/mL of betamethasone, prepared as follows. Transfer 1.0 mg of betamethasone from a portion of Ointment to a suitable glass centrifuge tube. Add 15.0 mL of *Diluent A* and mix with a vortex mixer to disperse the sample thoroughly. Add 35.0 mL of *Diluent B* and sonicate for 10 min with intermittent shaking. Centrifuge to obtain a clear supernatant and use the clear supernatant.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm. For *Identification B*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 100 μL

Autosampler temperature: 4°

**System suitability**Samples: *System suitability solution* and *Standard solution*[NOTE—See *Table 2* for relative retention times.]**Suitability requirements**Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*Tailing factor: NMT 2.0, *Standard solution*Relative standard deviation: NMT 1.0%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of betamethasone (C<sub>22</sub>H<sub>29</sub>FO<sub>5</sub>) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Betamethasone Valerate RS in the *Standard solution* (μg/mL) $C_U$  = nominal concentration of betamethasone in the *Sample solution* (μg/mL) $M_{r1}$  = molecular weight of betamethasone, 392.46 $M_{r2}$  = molecular weight of betamethasone valerate, 476.58<sub>▲USP40</sub>

Acceptance criteria: 90.0%–110.0%

**IMPURITIES****Add the following:****▲ ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, Diluent A, Diluent B, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 0.25 μg/mL each of USP Betamethasone RS, USP Betamethasone Valerate RS, and USP Betamethasone Valerate Related Compound A RS in Diluent B. Sonicate to dissolve if necessary.

**System suitability**Samples: *System suitability solution* and *Standard solution*[NOTE—See *Table 2* for relative retention times.]**Suitability requirements**Resolution: NLT 2.0 between betamethasone valerate and betamethasone valerate related compound A, *System suitability solution*Tailing factor: NMT 2.0, *Standard solution*Relative standard deviation: NMT 5.0%, *Standard solution***Analysis**Samples: *Sample solution* and *Standard solution*

Calculate the percentage of each specified degradation product in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each specified degradation product from the *Sample solution* $r_S$  = peak response of the corresponding USP Reference Standard from the *Standard solution* $C_S$  = concentration of the corresponding USP Reference Standard in the *Standard solution* (μg/mL) $C_U$  = nominal concentration of betamethasone in the *Sample solution* (μg/mL)

Calculate the percentage of each unspecified degradation product in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak response of each unspecified degradation product from the *Sample solution* $r_S$  = peak response of betamethasone valerate from the *Standard solution* $C_S$  = concentration of USP Betamethasone Valerate RS in the *Standard solution* (μg/mL) $C_U$  = nominal concentration of betamethasone in the *Sample solution* (μg/mL) $M_{r1}$  = molecular weight of betamethasone, 392.46 $M_{r2}$  = molecular weight of betamethasone valerate, 476.58Acceptance criteria: See *Table 2*. Disregard any impurity peak less than 0.1%.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Betamethasone	0.30	1.0
Betamethasone valerate	1.00	—
Betamethasone valerate related compound A	1.04	1.0
Any individual unspecified degradation product	—	1.0
Total degradation products	—	2.0

▲USP40

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): Meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

- **MINIMUM FILL** (755): Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers, and avoid exposure to excessive heat.

**Change to read:**

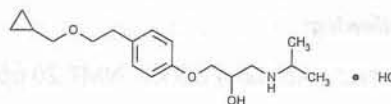
- **USP REFERENCE STANDARDS** (11)

▲USP Betamethasone RS<sub>▲USP40</sub>

USP Betamethasone Valerate RS

▲USP Betamethasone Valerate Related Compound A RS

9-Fluoro-11β,17-dihydroxy-16β-methyl-3,20-dioxopregna-1,4-dien-21-yl valerate.

C<sub>27</sub>H<sub>37</sub>FO<sub>6</sub> 476.58<sub>▲USP40</sub>**Betaxolol Hydrochloride**C<sub>18</sub>H<sub>29</sub>NO<sub>3</sub> · HCl

343.89



2-Propanol, 1-[4-[2-(cyclopropylmethoxy)ethyl]phenoxy]-3-[(1-methylethyl)amino]-, hydrochloride, (±)-;  
(±)-1-[p-[2-(Cyclopropylmethoxy)ethyl]phenoxy]-3-(isopropylamino)-2-propanol hydrochloride [63659-19-8].

**DEFINITION**

Betaxolol Hydrochloride contains NLT 98.0% and NMT 102.0% of betaxolol hydrochloride ( $C_{18}H_{29}NO_3 \cdot HCl$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191)  
Analysis: Proceed as directed for alkaloidal hydrochlorides.  
Acceptance criteria: Meets the requirements

**ASSAY**• **PROCEDURE**

**Buffer:** 0.025 M monobasic potassium phosphate containing 0.1% (w/v) of tetrabutyl ammonium bromide. Adjust with 0.025 M phosphoric acid to a pH of 3.0.

**Mobile phase:** Acetonitrile and *Buffer* (15:85)

**System suitability solution:** 2.0 mg/mL of USP Betaxolol Hydrochloride RS and 1.0 mg/mL of alprenolol hydrochloride in *Mobile phase*

**Standard solution:** 2.0 mg/mL of USP Betaxolol Hydrochloride RS in *Mobile phase*

**Sample solution:** 2.0 mg/mL of Betaxolol Hydrochloride in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 273 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for alprenolol and betaxolol are 0.9 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.0 between alprenolol and betaxolol

**Tailing factor:** NMT 2.0 for the alprenolol and betaxolol peaks

**Relative standard deviation:** NMT 1.0% for the betaxolol peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of betaxolol hydrochloride ( $C_{18}H_{29}NO_3 \cdot HCl$ ) in the portion of Betaxolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Betaxolol Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**Delete the following:**

- **HEAVY METALS, Method II** (231): NMT 20 ppm • (Official 1-

Jan-2018)

• **ORGANIC IMPURITIES**

**Buffer, Mobile phase, System suitability solution, Sample solution, and System suitability:** Proceed as directed in the *Assay*.

**Chromatographic system:** Proceed as directed in the *Assay*, except to use a run time of NLT 5 times the retention time of betaxolol.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Betaxolol Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each individual peak other than the main betaxolol peak

$r_T$  = sum of all the peak responses

Acceptance criteria: NMT 1.0% for the sum of all impurities

**SPECIFIC TESTS**• **PH** (791)

**Sample solution:** 20 mg/mL

Acceptance criteria: 4.5–6.5

• **LOSS ON DRYING** (731)

**Analysis:** Dry under vacuum at 65° for 2 h.

Acceptance criteria: NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Betaxolol Hydrochloride RS

**Betaxolol Ophthalmic Solution****DEFINITION**

Betaxolol Ophthalmic Solution is a sterile, aqueous, isotonic solution of Betaxolol Hydrochloride. It contains a suitable antimicrobial preservative. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of betaxolol ( $C_{18}H_{29}NO_3$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** Dissolve 7.1 g of anhydrous dibasic sodium phosphate in 800 mL of water, adjust with phosphoric acid to a pH of 3.0, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Standard solution:** 0.11 mg/mL of USP Betaxolol Hydrochloride RS in *Buffer*

**Sample solution:** Nominally 0.1 mg/mL of betaxolol in *Buffer* from Ophthalmic Solution

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV or diode array 280 nm. [NOTE—Use the diode array detector to perform *Identification test B*.]



Column: 4-mm × 25-cm; packing L1

Flow rate: 1.1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: 0.8–2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betaxolol ( $C_{18}H_{29}NO_3$ ) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of betaxolol in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of betaxolol, 307.43

$M_{r2}$  = molecular weight of betaxolol hydrochloride, 343.89

Acceptance criteria: 90.0%–110.0%

## IMPURITIES

### • ORGANIC IMPURITIES

**Mobile phase:** Add 5 mL of phosphoric acid to 990 mL of water. Adjust with 2 M ammonium hydroxide to a pH of 3.0, and dilute with water to 1000 mL. Prepare a mixture of this solution and acetonitrile (45:55). Dissolve 3 g of sodium dodecyl sulfate in 450 mL of the mixture.

**Standard solution:** 2.2 µg/mL of USP Betaxolol Hydrochloride RS in *Mobile phase*

**Sample solution:** Nominally equivalent to 0.2 mg/mL of betaxolol in *Mobile phase* from Ophthalmic Solution

**Chromatographic system**

(See *Chromatography* {621}, *System Suitability*.)

Mode: LC

Detector: 220 nm

Column: 4.6-mm × 25-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 5%

Tailing factor: NMT 2.5

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of betaxolol from the *Standard solution*

$C_S$  = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of betaxolol in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of betaxolol hydrochloride, 343.89

$M_{r2}$  = molecular weight of betaxolol, 307.43

Acceptance criteria

Single largest individual impurity: NMT 1%

Any other individual impurity: NMT 0.3%

## SPECIFIC TESTS

• **STERILITY TESTS** {71}: It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **PH** {791}: 4.0–8.0

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.

• **USP REFERENCE STANDARDS** {11}

USP Betaxolol Hydrochloride RS

## Betaxolol Tablets

### DEFINITION

Betaxolol Tablets contain an amount of Betaxolol Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betaxolol hydrochloride ( $C_{18}H_{29}NO_3 \cdot HCl$ ).

### IDENTIFICATION

• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** 0.025 M pH 6.0 ammonium phosphate buffer  
**Mobile phase:** *Buffer*, acetonitrile, and methanol (35:35:30)

**Diluent:** Acetonitrile and water (1:1)

**Standard solution:** 2 mg/mL of USP Betaxolol Hydrochloride RS in *Diluent*

**Sample solution:** Nominally 2 mg/mL of betaxolol hydrochloride prepared as follows. Place NLT 20 Tablets in a suitable volumetric flask with an appropriate amount of *Diluent*. Sonicate until the Tablets are disintegrated. Cool to room temperature, dilute with *Diluent* to volume, and filter. Use the clear filtrate.

**Chromatographic system**

(See *Chromatography* {621}, *System Suitability*.)

Mode: LC

Detector: UV 273 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 3.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of betaxolol hydrochloride ( $C_{18}H_{29}NO_3 \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Betaxolol Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of betaxolol hydrochloride in the *Sample solution* (mg/mL)



Acceptance criteria: 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Betaxolol Hydrochloride RS in Medium

Sample solutions: Sample per *Dissolution* (711). Dilute with Medium to a concentration that is similar to that of the Standard solution.

#### Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance of about 274 nm

Cell path: A 5-cm path length cell may be used for lower dosage levels.

Tolerances: NLT 80% (Q) of the labeled amount of betaxolol hydrochloride ( $C_{18}H_{29}NO_3 \cdot HCl$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905)

##### Procedure for content uniformity

Standard solution: 0.1 mg/mL of USP Betaxolol Hydrochloride RS in 0.1 N hydrochloric acid

Sample solution: Place 1 Tablet in a suitable volumetric flask to obtain a concentration of betaxolol hydrochloride, based on the labeled claim, of 0.1 mg/mL. Add an amount of 0.1 N hydrochloric acid equal to 70% of the volume of the flask. Shake by mechanical means until dissolved, dilute with 0.1 N hydrochloric acid to volume, and mix. Filter, and discard the first 20 mL of the filtrate.

#### Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance of about 274 nm

Cell path: 1 cm

Blank: 0.1 N hydrochloric acid

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of betaxolol hydrochloride ( $C_{18}H_{29}NO_3 \cdot HCl$ ) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the Sample solution

$A_S$  = absorbance of the Standard solution

$C_S$  = concentration of USP Betaxolol Hydrochloride RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of betaxolol hydrochloride in the Sample solution (mg/mL)

Acceptance criteria: Meet the requirements

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Label the Tablets to state both the content of the betaxolol active moiety and the content of betaxolol hydrochloride used in formulating them.

• **USP REFERENCE STANDARDS (11)**

USP Betaxolol Hydrochloride RS

1-Propanaminium, 2-[(aminocarbonyl)oxy]-N,N,N-trimethyl-, chloride, ( $\pm$ );  
( $\pm$ )-(2-Hydroxypropyl)trimethylammonium chloride carboxylate [590-63-6].

### DEFINITION

Bethanechol Chloride contains NLT 98.0% and NMT 101.5% of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ), calculated on the dried basis.

### IDENTIFICATION

• **A. INFRARED ABSORPTION (197M)**

• **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

• **C. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

### ASSAY

#### • PROCEDURE

Buffer: 29 mg/L of edetic acid in a solution prepared as follows. Transfer a portion of edetic acid to a suitable volumetric flask. Dissolve in water, using 50% of the final flask volume. Add 0.3 mL of nitric acid per L, and dilute with water to volume.

Mobile phase: Acetonitrile and Buffer (5:95)

System suitability solution: 0.1 mg/mL of bethanechol chloride in a solution prepared as follows. Transfer a portion of bethanechol chloride to a suitable volumetric flask. Add 4% of the final flask volume of 0.1 N sodium hydroxide, and allow to stand for 15 min. Add 4% of the final flask volume of 0.1 N hydrochloric acid. Dissolve in and dilute with Mobile phase to volume.

Standard solution: 0.1 mg/mL of USP Bethanechol Chloride RS in Mobile phase

Sample solution: 0.1 mg/mL of Bethanechol Chloride in Mobile phase

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: Conductivity

Column: 3.9-mm  $\times$  15.0-cm; packing L5S

Temperatures

Detector: 35°

Column: 30°

Flow rate: 1 mL/min

Injection volume: 25  $\mu$ L

#### System suitability

Samples: System suitability solution and Standard solution

[NOTE—See Table 1 for the relative retention times.]

#### Suitability requirements

Resolution: NLT 0.8 between desacetyl methacholine and bethanechol chloride, System suitability solution

Tailing factor: NMT 3.5, Standard solution

Relative standard deviation: NMT 3.0%, Standard solution

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) in the portion of Bethanechol Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

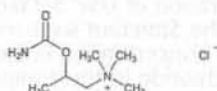
$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Bethanechol Chloride RS in the Standard solution (mg/mL)

$C_U$  = concentration of Bethanechol Chloride in the Sample solution (mg/mL)

## Bethanechol Chloride





Acceptance criteria: 98.0%–101.5% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

### Delete the following:

- **HEAVY METALS, Method I** (231)

**Test preparation:** Dissolve 667 mg of Bethanechol Chloride in 10 mL of water, add 2 mL of 1 N acetic acid, and dilute with water to 25 mL.

**Acceptance criteria:** NMT 30 ppm (Official 1-Jan-2018)

- **ORGANIC IMPURITIES**

**Buffer:** 0.48 g/L of methanesulfonic acid in water

**Mobile phase:** Acetonitrile and Buffer (5:95)

**System suitability solution:** 0.1 mg/mL of bethanechol chloride in a solution prepared as follows. Transfer a portion of bethanechol chloride to a suitable volumetric flask. Add 4% of the final flask volume of 0.1 N sodium hydroxide, and allow to stand for 15 min. Add 4% of the final flask volume of 0.1 N hydrochloric acid. Dissolve in and dilute with *Mobile phase* to volume.

**Standard solution:** 1 µg/mL of USP Bethanechol Chloride RS in *Mobile phase*

**Sample solution:** 0.1 mg/mL of Bethanechol Chloride in *Mobile phase*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Conductivity

**Column:** 3.9-mm × 15.0-cm; packing L55

**Temperatures**

**Detector:** 35°

**Column:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 50 µL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

### Suitability requirements

**Resolution:** NLT 0.8 between desacetyl methacholine and bethanechol chloride, *System suitability solution*

**Relative standard deviation:** NMT 10.0% for bethanechol chloride, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Bethanechol Chloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of any impurity from the *Sample solution*

$r_s$  = peak response of bethanechol chloride from the *Standard solution*

$C_s$  = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Bethanechol Chloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desacetyl methacholine <sup>a</sup>	0.9	1.2	1.0
Bethanechol chloride	1.0	—	—

<sup>a</sup> 2-Hydroxypropyltrimethyl ammonium chloride.

**Table 1 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	1.5

<sup>a</sup> 2-Hydroxypropyltrimethyl ammonium chloride.

### SPECIFIC TESTS

- **PH** (791)

**Sample solution:** 10 mg/mL of Bethanechol Chloride in water

**Acceptance criteria:** 5.5–6.5

- **LOSS ON DRYING** (731)

**Analysis:** Dry at 105° for 2 h.

**Acceptance criteria:** NMT 1.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS** (11)

USP Bethanechol Chloride RS

## Bethanechol Chloride Injection

### DEFINITION

Bethanechol Chloride Injection is a sterile solution of Bethanechol Chloride in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of bethanechol chloride (C<sub>7</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>2</sub>).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191): Meets the requirements

### ASSAY

- **PROCEDURE**

**Mobile phase:** 20 mM methanesulfonic acid

**Diluent:** 0.1 mg/mL of calcium chloride and 0.1 mg/mL of magnesium chloride in water

**System suitability solution:** 1 mg/mL of USP Bethanechol Chloride RS in solution prepared as follows. Transfer a portion of USP Bethanechol Chloride RS to a suitable volumetric flask. Add 60% of the final volume of water, 8% of the final volume of *Diluent*, and 2% of the final volume of 0.1 N of sodium hydroxide. Dilute with water to volume.

**Standard solution:** 1.0 mg/mL of USP Bethanechol Chloride RS in water

**Sample solution:** Nominally 1.0 mg/mL of bethanechol chloride from a volume of Injection in water

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Conductivity

**Column:** 4-mm × 25-cm; packing L53

**Flow rate:** 1 mL/min

**Injection volume:** 50 µL

### System suitability

**Sample:** *System suitability solution*

[NOTE—See *Table 1* for the relative retention times.]

### Suitability requirements

**Resolution:** NLT 2.0 between the calcium ion and desacetyl methacholine

**Tailing factor:** NMT 4.5 for bethanechol chloride  
**Relative standard deviation:** NMT 2.0% for bethanechol chloride



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) in each mL of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0%

**IMPURITIES**• **ORGANIC IMPURITIES**

**Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of desacetyl methacholine in each mL of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of desacetyl methacholine from the *Sample solution*  
 $r_S$  = peak response of bethanechol chloride from the *Standard solution*  
 $C_S$  = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of bethanechol chloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sodium <sup>a</sup>	1.0	—
Magnesium <sup>a</sup>	1.4	—
Calcium <sup>a</sup>	1.6	—
Desacetyl methacholine <sup>b</sup>	2.0	4.0
Bethanechol chloride	2.8	—

<sup>a</sup> Included for identification purposes only.

<sup>b</sup> 2-Hydroxypropyltrimethyl ammonium chloride.

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 25.0 USP Endotoxin Units/mg of bethanechol chloride
- **pH** (791): 5.5–7.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS** (11)

USP Bethanechol Chloride RS  
USP Endotoxin RS

**Bethanechol Chloride Compounded Oral Solution****DEFINITION**

Bethanechol Chloride Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ).

Prepare Bethanechol Chloride Compounded Oral Solution 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Bethanechol Chloride	500 mg
Vehicle for Oral Solution (regular or sugar-free), NF, a sufficient quantity to make	100 mL

Add *Bethanechol Chloride* powder and about 20 mL of *Vehicle for Oral Solution* to a mortar, and mix. Add the *Vehicle for Oral Solution* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle for Oral Solution* to bring to final volume, and mix well.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Acetonitrile and water (33:67)

**Standard solution:** 500 µg/mL of USP Bethanechol Chloride RS in *Mobile phase*

**Sample solution:** Agitate the container of Oral Solution for 30 min on a rotating mixer, remove a 10-mL sample, and store in a clear glass vial at –70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Dilute a suitable volume of Oral Solution with *Mobile phase* to obtain a nominal concentration of 500 µg/mL of bethanechol chloride.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L11

**Flow rate:** 0.7 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time of bethanechol chloride is about 3 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 3.1% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Bethanechol Chloride RS in the *Standard solution* (µg/mL)  
 $C_U$  = nominal concentration of bethanechol chloride in the *Sample solution* (µg/mL)



Acceptance criteria: 90.0%–110.0%

### SPECIFIC TESTS

- **PH** (791): 3.9–4.9

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at room temperature or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded when stored at room temperature or in a refrigerator
- **LABELING:** Label it to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** (11)  
USP Bethanechol Chloride RS

## Bethanechol Chloride Compounded Oral Suspension

### DEFINITION

Bethanechol Chloride Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ).

Prepare Bethanechol Chloride Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Bethanechol Chloride	500 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

If using *Bethanechol Chloride* tablets, add to a suitable mortar and comminute to a fine powder, or add the *Bethanechol Chloride* powder to the mortar. Add about 20 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to final volume, and mix well.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Acetonitrile and water (33:67)

**Standard solution:** 500 µg/mL of USP Bethanechol Chloride RS in *Mobile phase*

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 10-mL sample, and store in a clear glass vial at  $-70^\circ$  until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Dilute a suitable volume of Oral Suspension with *Mobile phase* to obtain a nominal concentration of 500 µg/mL of bethanechol chloride.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L11

**Flow rate:** 0.7 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for bethanechol chloride is about 3 min.]

#### Suitability requirements

**Relative standard deviation:** NMT 3.1% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) in the volume of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bethanechol Chloride RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of bethanechol chloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

### SPECIFIC TESTS

- **PH** (791): 3.9–4.9

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at room temperature, or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the day on which it was compounded when stored at room temperature, or in a refrigerator
- **LABELING:** Label it to state that it is to be well shaken, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** (11)  
USP Bethanechol Chloride RS

## Bethanechol Chloride Tablets

### DEFINITION

Bethanechol Chloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197M)

**Sample:** Nominally 100 mg of bethanechol chloride from a suitable portion of pulverized Tablets prepared as follows. Pulverize a portion of Tablets equivalent to 100 mg of bethanechol chloride. Add 15 mL of ether, and allow to digest for 15 min. Decant the ether, again extract the residue with 10 mL of ether, and discard the ether extracts. Add 30 mL of alcohol to the residue. Shake for 10 min, and allow to stand for 1 h with frequent agitation. Filter with suction, and evaporate the filtrate on a steam bath to dryness: the bethanechol chloride so obtained is recrystallized from alcohol and dried at  $105^\circ$  for 2 h.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** 29 mg/L of edetic acid in solution prepared as follows. Transfer a portion of edetic acid to a suitable volumetric flask. Dissolve with water, using 50% of the final volume. Add 0.3 mL of nitric acid per L, and dilute with water to volume.

**Mobile phase:** Acetonitrile and *Buffer* (5:95)

**System suitability solution:** 0.1 mg/mL of bethanechol chloride in solution prepared as follows. Transfer a portion of bethanechol chloride to a suitable volumetric flask. Add 4% of the final volume of 0.1 N sodium hydroxide, and allow to stand for 15 min. Add 4% of the final volume of 0.1 N hydrochloric acid. Dissolve in and dilute with *Mobile phase* to volume.



**Standard solution:** 0.1 mg/mL of USP Bethanechol Chloride RS in *Mobile phase*

**Sample solution:** Nominally 0.1 mg/mL of bethanechol chloride from a suitable amount of powdered Tablets in solution prepared as follows. Add a portion of fine powder, equivalent to 1 Tablet, from NLT 20 Tablets to a suitable volumetric flask. Dissolve in *Mobile phase*, using 60%–70% of the final volume. Sonicate for 20 min. Shake by mechanical means for 15 min. Dilute with *Mobile phase* to volume, and mix. Allow to stand for 10 min, and pass through a 1- $\mu$ m glass filter, discarding the first 3 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Conductivity

**Column:** 3.9-mm  $\times$  15.0-cm; packing L5S

#### Temperatures

**Detector:** 35°

**Column:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 0.8 between desacetyl methacholine and bethanechol chloride, *System suitability solution*

**Tailing factor:** NMT 3.5, *Standard solution*

**Relative standard deviation:** NMT 3.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Buffer, Mobile phase, and System suitability solution:**

Proceed as directed in the *Assay*.

**Standard solution:** (L/900) mg/mL of USP Bethanechol Chloride RS in *Medium*, where L is the label claim in mg/Tablet

**Sample solution:** A portion of solution under test

**Chromatographic system and System suitability:** Proceed as directed in the *Assay*, except for the following parameters:

#### Injection volumes

**For the System suitability solution:** 50  $\mu$ L

**For the Standard solution and Sample solution:** 100  $\mu$ L

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount (Q) of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)

V = volume of the *Medium*, 900 mL

L = label claim (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of bethanechol chloride ( $C_7H_{17}ClN_2O_2$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

### IMPURITIES

#### • ORGANIC IMPURITIES

**Buffer:** 0.48 g/L of methanesulfonic acid in water

**Mobile phase:** Acetonitrile and *Buffer* (5:95)

**System suitability solution:** 0.1 mg/mL of bethanechol chloride in solution prepared as follows. Transfer the bethanechol chloride to a suitable volumetric flask. Add 4% of the final volume of 0.1 N sodium hydroxide, and allow to stand for 15 min. Add 4% of the final volume of 0.1 N hydrochloric acid. Dissolve in and dilute with *Mobile phase* to volume.

**Standard solution:** 1  $\mu$ g/mL of USP Bethanechol Chloride RS in *Mobile phase*

**Sample solution:** Nominally 0.1 mg/mL of bethanechol chloride from a suitable amount of powdered Tablets in solution prepared as follows. Add a portion of fine powder, equivalent to 1 Tablet, from NLT 20 Tablets to a suitable volumetric flask. Dissolve in *Mobile phase*, using 60%–70% of the final volume. Sonicate for 20 min. Shake by mechanical means for 15 min. Dilute with *Mobile phase* to volume, and mix. Allow to stand for 10 min, and pass through a 1- $\mu$ m glass filter, discarding the first 3 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Conductivity

**Column:** 3.9-mm  $\times$  15.0-cm; packing L5S

#### Temperatures

**Detector:** 35°

**Column:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 0.8 between desacetyl methacholine and bethanechol chloride, *System suitability solution*

**Relative standard deviation:** NMT 10.0% for bethanechol chloride, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for any impurity in the *Sample solution*

$r_S$  = peak response of bethanechol chloride from the *Standard solution*

$C_S$  = concentration of USP Bethanechol Chloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bethanechol chloride in the *Sample solution* (mg/mL)

F = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*.



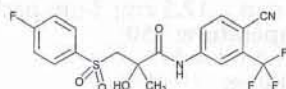
Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desacetyl methacholine <sup>a</sup>	0.9	1.2	1.0
Bethanechol chloride	1.0	—	—
Any unspecified degradation product	—	1.0	0.2
Total impurities	—	—	1.5

<sup>a</sup> 2-Hydroxypropyltrimethyl ammonium chloride.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
USP Bethanechol Chloride RS

**Bicalutamide**

$C_{18}H_{14}F_4N_2O_4S$  430.37  
Propanamide, N-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-, (±)-; (±)-4'-Cyano-α,α,α-trifluoro-3-[(p-fluorophenyl)sulfonyl]-2-methyl-m-lactotoluidide [90357-06-5].

**DEFINITION**

Bicalutamide contains NLT 98.0% and NMT 102.0% of  $C_{18}H_{14}F_4N_2O_4S$ , calculated on the anhydrous and solvent-free basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Solution A:** 0.01% (v/v) of trifluoroacetic acid in water

**Solution B:** 0.01% (v/v) of trifluoroacetic acid in acetonitrile

**Mobile phase:** *Solution A* and *Solution B* (52:48)

**Diluent:** *Solution A* and *Solution B* (1:2)

**System suitability solution:** 5 µg/mL of USP Bicalutamide Related Compound A RS and 50 µg/mL of USP Bicalutamide RS in *Diluent*

**Standard solution:** 0.05 mg/mL of USP Bicalutamide RS in *Diluent*

**Sample solution:** 0.05 mg/mL of Bicalutamide in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 270 nm

**Column:** 4.0-mm × 10-cm; 3-µm packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for bicalutamide related compound A isomer A and bicalutamide related

compound A isomer B are 0.75 and 0.78, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between bicalutamide related compound A isomer B and bicalutamide, *System suitability solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{18}H_{14}F_4N_2O_4S$  in the portion of Bicalutamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION (281):** NMT 0.1%

**Delete the following:**

- **HEAVY METALS, Method II (231):** NMT 10 ppm (Official 1-Jan-2018)

**Organic Impurities**• **PROCEDURE**

**Solution A, Solution B, Diluent, System suitability solution, and Chromatographic system:** Proceed as directed in the Assay.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	67	33
16.5	67	33
26.5	40	60
32.5	5	95
32.6	67	33
35	67	33

**Standard solution:** 1 µg/mL of USP Bicalutamide RS in *Diluent*

**Sample solution:** 1 mg/mL of Bicalutamide in *Diluent*

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution 1:** NLT 0.8 between bicalutamide related compound A isomer A and bicalutamide related compound A isomer B

**Resolution 2:** NLT 8.5 between bicalutamide related compound A isomer B and bicalutamide

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Bicalutamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak area of each impurity from the *Sample solution*

$r_S$  = peak area of bicalutamide from the *Standard solution*

$C_S$  = concentration of bicalutamide in the *Standard solution* (mg/mL)

$C_U$  = concentration of Bicalutamide in the *Sample solution* (mg/mL)



F = relative response factor (see *Impurity Table 1*)  
 Acceptance criteria  
 Individual impurities: See *Impurity Table 1*.  
 Total impurities: NMT 0.5%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bicalutamide aminobenzonitrile <sup>a</sup>	0.30	1.4	0.1
Bicalutamide related compound A isomer A <sup>b</sup>	0.64	1.0	0.1
Bicalutamide related compound A isomer B <sup>c</sup>	0.67	1.0	0.1
Desfluoro bicalutamide <sup>c</sup>	0.83	1.1	0.2
2-Fluoro bicalutamide <sup>d</sup>	0.94	1.0	0.2
Bicalutamide	1.00	—	—
Deoxybicalutamide <sup>e</sup>	1.33	1.0	0.2
Bicalutamide sulfide <sup>f</sup>	1.56	1.0	0.1
Any unspecified impurity	—	1.0	0.1

<sup>a</sup> 4-Amino-2-(trifluoromethyl)benzonitrile.

<sup>b</sup> N-[4-Cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfinyl]-2-hydroxy-2-methylpropanamide.

<sup>c</sup> N-[4-Cyano-3-(trifluoromethyl)phenyl]-2-hydroxy-2-methyl-3-(phenylsulfonyl)propanamide.

<sup>d</sup> N-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(2-fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamide.

<sup>e</sup> N-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylsulfonyl)-2-methylpropanamide.

<sup>f</sup> N-[4-Cyano-3-(trifluoromethyl)phenyl]-3-(4-fluorophenylthio)-2-hydroxy-2-methylpropanamide.

## SPECIFIC TESTS

• **WATER DETERMINATION**, *Method 1* (921): NMT 0.2%

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers, and store at room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Bicalutamide RS
  - USP Bicalutamide Related Compound A RS
  - [N-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfinyl]-2-hydroxy-2-methylpropanamide] (C<sub>18</sub>H<sub>14</sub>F<sub>4</sub>N<sub>2</sub>O<sub>3</sub>S 414.37)

## Bicalutamide Tablets

### DEFINITION

Bicalutamide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bicalutamide (C<sub>18</sub>H<sub>14</sub>F<sub>4</sub>N<sub>2</sub>O<sub>4</sub>S).

### IDENTIFICATION

- **A**. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase**: Tetrahydrofuran, acetonitrile, and water (20:15:65)

**System suitability stock solution**: 0.8 mg/mL of USP Bicalutamide RS and 0.4 mg/mL of USP Bicalutamide Related Compound B RS in tetrahydrofuran

**System suitability solution**: 0.04 mg/mL of USP Bicalutamide RS and 0.02 mg/mL of USP Bicalutamide Related Compound B RS in *Mobile phase* from the *System suitability stock solution*

**Standard stock solution**: 0.8 mg/mL of USP Bicalutamide RS in tetrahydrofuran

**Standard solution**: 0.04 mg/mL of USP Bicalutamide RS in *Mobile phase* from the *Standard stock solution*

**Sample stock solution**: 0.5 mg/mL of bicalutamide in tetrahydrofuran prepared as follows. Transfer equivalent to 50 mg of bicalutamide from finely powdered Tablets (NLT 20) into a 100-mL volumetric flask. Add 50 mL of tetrahydrofuran, and sonicate for NLT 10 min to complete dissolution. Allow to cool to room temperature, and dilute with tetrahydrofuran to volume. Pass through a suitable filter of 0.45-μm pore size.

**Sample solution**: 0.04 mg/mL of bicalutamide in *Mobile phase* from the *Sample stock solution*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode**: LC

**Detector**: UV 270 nm

**Column**: 5-mm × 12.5-cm; 3-μm packing L1

**Column temperature**: 50°

**Flow rate**: 1.5 mL/min

**Injection volume**: 10 μL

### System suitability

**Sample**: *System suitability solution*

[NOTE—The relative retention times for bicalutamide and bicalutamide related compound B are 1.0 and 1.1, respectively.]

### Suitability requirements

**Resolution**: Greater than 1.9 between bicalutamide and bicalutamide related compound B

**Tailing factor**: Less than 1.3 for bicalutamide

**Relative standard deviation**: NMT 2.0% for bicalutamide

### Analysis

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bicalutamide (C<sub>18</sub>H<sub>14</sub>F<sub>4</sub>N<sub>2</sub>O<sub>4</sub>S) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bicalutamide in the *Sample solution* (mg/mL)

**Acceptance criteria**: 90.0%–110.0%

## PERFORMANCE TESTS

### DISSOLUTION (711)

#### Test 1

**Medium**: 1.0% w/v sodium lauryl sulfate in water; 1000 mL

**Apparatus 2**: 50 rpm

**Time**: 45 min

**Standard solution**: 0.05 mg/mL of USP Bicalutamide RS in *Medium* prepared as follows. Transfer USP Bicalutamide RS to a suitable volumetric flask, dissolve in tetrahydrofuran equivalent to 1% of the final volume, and dilute with *Medium* to volume.

**Sample solution**: Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size.



**Instrumental conditions**(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 270 nm

Blank: Medium

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of bicalutamide ( $C_{18}H_{14}F_4N_2O_4S$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

 $A_U$  = absorbance of the *Sample solution* $A_S$  = absorbance of the *Standard solution* $C_S$  = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL) $V$  = volume of *Medium* (mL) $L$  = label claim (mg/Tablet)Tolerances: NLT 80% (Q) of the labeled amount of bicalutamide ( $C_{18}H_{14}F_4N_2O_4S$ ) is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that the product meets USP *Dissolution* Test 2.

Medium, Apparatus 2, Time, Standard solution, Sample solution, and Instrumental conditions: Proceed as directed for Test 1.

Tolerances: NLT 75% (Q) of the labeled amount of bicalutamide ( $C_{18}H_{14}F_4N_2O_4S$ ) is dissolved.• **UNIFORMITY OF DOSAGE UNITS (905)****Procedure for content uniformity**

Diluent: 10 mg/mL of sodium lauryl sulfate in water

Standard solution: 0.05 mg/mL of USP Bicalutamide RS in *Diluent*. [NOTE—Dissolve USP Bicalutamide RS in a minimum volume of tetrahydrofuran before dilution with *Diluent*.]Sample stock solution: Transfer 1 Tablet to a 100-mL volumetric flask. Add 10 mL of water, and sonicate for approximately 30 min. Add 80 mL of tetrahydrofuran, and sonicate for 30 min to complete dissolution of bicalutamide. Allow to cool to room temperature, and dilute with tetrahydrofuran to volume. Pass a portion of the solution through a suitable filter of 0.45- $\mu$ m pore size.Sample solution: Transfer 10.0 mL of the *Sample stock solution* into a 100-mL volumetric flask, and dilute with *Diluent* to volume.**Instrumental conditions**(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 270 nm

Blank: *Diluent***Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of bicalutamide ( $C_{18}H_{14}F_4N_2O_4S$ ) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

 $A_U$  = absorbance of the *Sample solution* $A_S$  = absorbance of the *Standard solution* $C_S$  = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of bicalutamide in the *Sample solution* (mg/mL)

Acceptance criteria: Meet the requirements

**IMPURITIES**• **LIMIT OF 4-AMINO-2-(TRIFLUOROMETHYL)BENZONITRILE**

Mobile phase and System suitability solution: Proceed as directed in the Assay.

Standard stock solution: 0.2 mg/mL of USP Bicalutamide RS in tetrahydrofuran

Standard solution: 0.02 mg/mL of USP Bicalutamide RS in *Mobile phase* from the *Standard stock solution*Sample solution: Transfer equivalent to 50 mg of bicalutamide from powdered Tablets (NLT 20) to a 25-mL volumetric flask. Add 2 mL of tetrahydrofuran, and allow to stand for 5 min. Add 20 mL of *Mobile phase*, sonicate for 10 min, and allow to cool to room temperature. Dilute with *Mobile phase* to volume, and pass through a suitable filter of 0.2- $\mu$ m pore size.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 5-mm  $\times$  12.5-cm; 3- $\mu$ m packing L1

Column temperature: 50°

Flow rate: 1.5 mL/min

Injection volume: 10  $\mu$ L**System suitability**Sample: *System suitability solution*

[NOTE—The relative retention times of 4-amino-2-(trifluoromethyl)benzonitrile, bicalutamide, and bicalutamide related compound B are about 0.4, 1.0 and about 1.1, respectively.]

**Suitability requirements**

Resolution: Greater than 1.9 between bicalutamide and bicalutamide related compound B

Tailing factor: Less than 1.3 for bicalutamide

Relative standard deviation: NMT 2.0% for bicalutamide

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of 4-amino-2-(trifluoromethyl)benzonitrile in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak area of 4-amino-2-(trifluoromethyl)benzonitrile from the *Sample solution* $r_S$  = peak area of bicalutamide from the *Standard solution* $C_S$  = concentration of USP Bicalutamide RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of bicalutamide in the *Sample solution* (mg/mL) $F$  = relative response factor of 4-amino-2-(trifluoromethyl)benzonitrile, 1.4

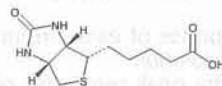
Acceptance criteria: NMT 0.1%

**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.• **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.• **USP REFERENCE STANDARDS (11)**

USP Bicalutamide RS

USP Bicalutamide Related Compound B RS

(RS)-N-(4-Cyano-3-(trifluoromethyl)phenyl)-3-(3-fluorophenylsulfonyl)-2-hydroxy-2-methylpropanamide.

 $C_{18}H_{14}F_4N_2O_4S$  430.37**Biotin** $C_{10}H_{16}N_2O_3S$  244.311*H*-Thieno[3,4-*d*]imidazole-4-pentanoic acid, hexahydro-2-oxo-, [3*aS*-(3*a* $\alpha$ ,4*\beta*,6*a* $\alpha$ )-]; (3*aS*,4*S*,6*aR*)-Hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-valeric acid [58-85-5].



**DEFINITION**

Biotin contains NLT 97.5% and NMT 102.0% of biotin ( $C_{10}H_{16}N_2O_3S$ ).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B.** It meets the requirements in *Specific Tests for Optical Rotation* (781S), *Specific Rotation*.
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer solution:** Dissolve 1 g of sodium perchlorate monohydrate in 500 mL of water, add 1 mL of phosphoric acid, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile and *Buffer solution* (8.5: 91.5)

**Diluent:** Acetonitrile and water (1:4)

**Standard solution:** 0.1 mg/mL of USP Biotin RS in *Diluent*. Sonicate if necessary to dissolve.

**Sample solution:** 0.1 mg/mL of Biotin in *Diluent*. Sonicate if necessary to dissolve.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm  $\times$  15-cm; 3- $\mu$ m packing L7

**Flow rate:** 1.2 mL/min

**Injection volume:** 50  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of biotin ( $C_{10}H_{16}N_2O_3S$ ) in the portion of Biotin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Biotin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Biotin in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.5%–102.0%

**IMPURITIES**• **RELATED COMPOUNDS**

**Buffer solution, Mobile phase, Diluent, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Sample:** *Sample solution*

Measure the peak responses of the *Sample solution*.

Calculate the percentage of each impurity in the portion of Biotin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_T$  = sum of the peak responses of all the peaks from the *Sample solution*

**Acceptance criteria**

**Individual impurity:** NMT 1.0%

**Total impurities:** NMT 2.0%

**SPECIFIC TESTS**• **OPTICAL ROTATION, Specific Rotation** (781S)

**Sample solution:** 20 mg/mL in 0.1 N sodium hydroxide

**Acceptance criteria:** +89° to +93°

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Store in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Biotin RS

**Biotin Capsules****DEFINITION**

Biotin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of biotin ( $C_{10}H_{16}N_2O_3S$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer solution:** Dissolve 1 g of sodium perchlorate monohydrate in 500 mL of water, add 1 mL of phosphoric acid, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile and *Buffer solution* (8.5: 91.5)

**Diluent:** Acetonitrile and water (1:4)

**Standard solution:** 0.05 mg/mL of USP Biotin RS in *Diluent*. Sonicate, if necessary, to dissolve.

**Sample solution:** Weigh NLT 20 Capsules in a tared weighing bottle. Open the Capsules, without the loss of shell material, and transfer the contents to a 100-mL beaker. Remove any contents adhering to the empty shells by washing, if necessary, with several portions of ether. Discard the washings, and dry the Capsule shells with the aid of a current of dry air until the odor of ether is no longer perceptible. Weigh the empty Capsule shells in the tared weighing bottle, and calculate the average net weight per Capsule. Transfer a portion of the Capsule contents, equivalent to a nominal amount of 5 mg of biotin to a 100-mL volumetric flask, add 60 mL of water, and shake in a water bath at 65° for 20 min. Sonicate for 5 min, shake by mechanical means for 15 min, and cool to room temperature. Add 20 mL of acetonitrile, dilute with water to volume, and filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm  $\times$  15-cm; 3- $\mu$ m packing L7

**Flow rate:** 1.2 mL/min

**Injection volume:** 50  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of biotin ( $C_{10}H_{16}N_2O_3S$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Biotin RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of biotin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

**Buffer solution:** 0.02 N anhydrous disodium hydrogen phosphate adjusted with phosphoric acid to a pH of 7.4  
**Medium:** *Buffer solution*; 500 mL

**Apparatus 1:** 100 rpm

**Time:** 1 h

**Standard solution:** Dissolve a suitable amount of USP Biotin RS in *Buffer solution* to obtain a concentration similar to that expected in the *Sample solution*.

**Sample solution:** Withdraw a portion of the solution under test, pass through a suitable filter, and use the pooled sample as the test specimen.

**Analysis:** Proceed as directed in the *Assay*, making any necessary adjustments.

Calculate the percentage of the labeled amount of biotin ( $C_{10}H_{16}N_2O_3S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times V/L) \times 100$$

- $r_U$  = peak area from the *Sample solution*  
 $r_S$  = peak area from the *Standard solution*  
 $C_S$  = concentration of USP Biotin RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $V$  = volume of *Medium*, 500 mL  
 $L$  = labeled amount of biotin ( $\mu\text{g/Capsule}$ )

**Tolerances:** NLT 75% (Q) of the labeled amount of biotin ( $C_{10}H_{16}N_2O_3S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Biotin RS

## Biotin Tablets

### DEFINITION

Biotin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of biotin ( $C_{10}H_{16}N_2O_3S$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer solution:** Dissolve 1 g of sodium perchlorate monohydrate in 500 mL of water, add 1 mL of phosphoric acid, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile and *Buffer solution* (8.5:91.5)

**Diluent:** Acetonitrile and water (1:4)

**Standard solution:** 0.05 mg/mL of USP Biotin RS in *Diluent*. Sonicate, if necessary, to dissolve.

**Sample solution:** Transfer a portion equivalent to 5 mg of biotin from NLT 30 finely powdered Tablets to a 100-mL volumetric flask, add 60 mL of water, and

shake in a water bath at 65° for 20 min. Sonicate for 5 min, shake by mechanical means for 15 min, and cool to room temperature. Add 20 mL of acetonitrile, dilute with water to volume, and filter.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm  $\times$  15-cm; 3- $\mu\text{m}$  packing L7

**Flow rate:** 1.2 mL/min

**Injection volume:** 50  $\mu\text{L}$

### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of biotin ( $C_{10}H_{16}N_2O_3S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Biotin RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of biotin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

**Buffer solution:** 0.02 N anhydrous disodium hydrogen phosphate adjusted with phosphoric acid to a pH of 7.4  
**Medium:** *Buffer solution*; 500 mL

**Apparatus 2:** 75 rpm

**Time:** 1 h

**Standard solution:** Dissolve a suitable amount of USP Biotin RS in *Buffer solution* to obtain a concentration similar to that expected in the *Sample solution*.

**Sample solution:** Withdraw a portion of the solution under test, pass through a suitable filter, and use the pooled sample as the test specimen.

**Analysis:** Proceed as directed in the *Assay*, making any necessary adjustments.

Calculate the percentage of the labeled amount of biotin ( $C_{10}H_{16}N_2O_3S$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S \times V/L) \times 100$$

- $r_U$  = peak area from the *Sample solution*  
 $r_S$  = peak area from the *Standard solution*  
 $C_S$  = concentration of USP Biotin RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $V$  = volume of *Medium*, 500 mL  
 $L$  = labeled amount of biotin ( $\mu\text{g/Tablet}$ )

**Tolerances:** NLT 75% (Q) of the labeled amount of biotin ( $C_{10}H_{16}N_2O_3S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

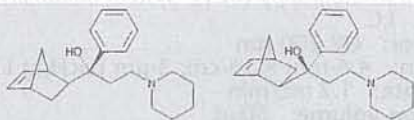
## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Biotin RS



Delete the following:

## Biperiden



$C_{21}H_{29}NO$  311.46  
1-Piperidinepropanol,  $\alpha$ -bicyclo[2.2.1]hept-5-en-2-yl- $\alpha$ -phenyl-;  
 $\alpha$ -5-Norbornen-2-yl- $\alpha$ -phenyl-1-piperidinepropanol  
[514-65-8].

### DEFINITION

Biperiden contains NLT 98.0% and NMT 101.0% of biperiden ( $C_{21}H_{29}NO$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B. ULTRAVIOLET ABSORPTION (197U)**

Analytical wavelength: 257 nm

Sample solution: 0.9 mg/mL of Biperiden in solution prepared as follows. Transfer a suitable amount of Biperiden to an appropriate volumetric flask. Add 0.5% of the flask volume of lactic acid, and dilute with water to volume.

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

- **C.**

Sample: 20 mg of Biperiden

Analysis: Dissolve the Sample in 5 mL of phosphoric acid.

Acceptance criteria: A green color is produced.

- **D.**

Sample solution: Dissolve 200 mg of Biperiden in 80 mL of water with the aid of 0.5 mL of 3 N hydrochloric acid. The resulting solution may be warmed, if necessary, to promote dissolution. Allow the resulting solution to cool.

Analysis

Part 1: To 5 mL of Sample solution add 1 drop of hydrochloric acid and several drops of mercuric chloride TS.

Part 2: To 5 mL of Sample solution add bromine TS dropwise.

Acceptance criteria: The acceptance criteria for both Part 1 and Part 2 must be met.

Part 1: A white precipitate is formed.

Part 2: A yellow precipitate forms, which redissolves on shaking; and finally, upon the addition of more bromine TS, a permanent precipitate is formed.

### ASSAY

- **PROCEDURE**

Sample: 500 mg of Biperiden

Blank: 20 mL of benzene

Titrimetric system

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Visual

Analysis: Dissolve the Sample in 20 mL of benzene, and add 2 drops of crystal violet TS. Titrate with Titrant to a blue endpoint. Perform a blank titration, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.15 mg of biperiden ( $C_{21}H_{29}NO$ ).

Acceptance criteria: 98.0%–101.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

- **ORDINARY IMPURITIES (466)**

Standard solution: Use methanol as the solvent.

Test solution: Use methanol as the solvent.

Eluant: A mixture of methanol and ammonium hydroxide (100:1.5)

Visualization: 17

Acceptance criteria: Meets the requirements

### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE, Class I (741):**

112°–116°

- **LOSS ON DRYING (731)**

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 1.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

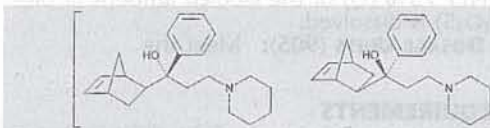
- **USP REFERENCE STANDARDS (11)**

USP Biperiden RS

▲USP40

Delete the following:

## Biperiden Hydrochloride



$C_{21}H_{29}NO \cdot HCl$  347.92  
1-Piperidinepropanol,  $\alpha$ -bicyclo[2.2.1]hept-5-en-2-yl- $\alpha$ -phenyl-, hydrochloride;  
 $\alpha$ -5-Norbornen-2-yl- $\alpha$ -phenyl-1-piperidinepropanol hydrochloride [1235-82-1].

### DEFINITION

Biperiden Hydrochloride contains NLT 98.0% and NMT 101.0% of biperiden hydrochloride ( $C_{21}H_{29}NO \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B. ULTRAVIOLET ABSORPTION (197U)**

Analytical wavelength: 257 nm

Sample solution: 1 mg/mL of Biperiden Hydrochloride in methanol

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

- **C.**

Sample: 20 mg of Biperiden Hydrochloride

Analysis: Dissolve the Sample in 5 mL of phosphoric acid.

Acceptance criteria: A green color is produced.

- **D.**

Sample solution: 2 mg/mL of Biperiden Hydrochloride in water

Analysis: To a 5-mL portion of the Sample solution add bromine TS dropwise.

Acceptance criteria: A yellow precipitate, which dissolves on shaking, is formed. Addition of more bromine TS produces a precipitate that does not dissolve on shaking.



### • E. IDENTIFICATION TESTS—GENERAL, Chloride (191)

**Sample solution:** 2 mg/mL of Biperiden Hydrochloride in water

**Analysis:** Proceed as directed in the chapter using a 5-mL portion of the *Sample solution*.

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Sample:** 500 mg of Biperiden Hydrochloride

**Blank:** 80 mL of glacial acetic acid

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 80 mL of glacial acetic acid, warming slightly if necessary, to effect solution.

Cool, add 1 drop of crystal violet TS and 10 mL of mercuric acetate TS. Titrate with *Titrant* to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 34.79 mg of biperiden hydrochloride ( $C_{21}H_{29}NO \cdot HCl$ ).

**Acceptance criteria:** 98.0%–101.0% on the dried basis

### IMPURITIES

#### • ORDINARY IMPURITIES (466)

**Standard solution:** Use methanol as the solvent.

**Test solution:** Use methanol as the solvent.

**Eluent:** A mixture of methanol and ammonium hydroxide (100:1.5)

**Visualization:** 17

**Acceptance criteria:** Meets the requirements

### SPECIFIC TESTS

#### • LOSS ON DRYING (731)

**Analysis:** Dry at 105° for 3 h.

**Acceptance criteria:** NMT 0.5%

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

#### • USP REFERENCE STANDARDS (11)

USP Biperiden Hydrochloride RS

▲ USP40

**Delete the following:**

## ▲Biperiden Hydrochloride Tablets

### DEFINITION

Biperiden Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of  $C_{21}H_{29}NO \cdot HCl$ .

### IDENTIFICATION

#### • THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Standard solution:** Dissolve 10 mg of USP Biperiden Hydrochloride RS in 5 mL of water, mix, and sonicate to disperse the powder. Add 5 mL of methanol to the flask, mix, and sonicate for 15 min. Filter the solution into a separator, add 2 mL of 1 N sodium hydroxide and 10 mL of chloroform, and shake for 3 min. Filter the chloroform layer into a stoppered flask, and use the chloroform filtrate.

**Sample solution:** To a quantity of finely powdered Tablets, equivalent to 10 mg of biperiden hydrochloride, add 5 mL of water, mix, and sonicate to disperse the powder. Add 5 mL of methanol to the flask, mix, and sonicate for 15 min. Filter the solution into a separator,

add 2 mL of 1 N sodium hydroxide and 10 mL of chloroform, and shake for 3 min. Filter the chloroform layer into a stoppered flask, and use the chloroform filtrate.

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture. Condition by heating the plate at 105° for 1 h and allowing to cool.

**Application volume:** 20  $\mu$ L

**Developing solvent system:** Methanol and ammonium hydroxide (100:1.5)

**Visualization:** Iodine vapor, 10 min

**Analysis:** Separately apply the *Sample solution* and the *Standard solution* to the chromatographic plate. Allow the applications to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by exposing the plate for 10 min to iodine vapors in a preequilibrated closed chamber, on the bottom of which there are iodine crystals.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

### ASSAY

#### • PROCEDURE

**Solution A:** 38 g/L of monobasic sodium phosphate and 2 g/L of anhydrous dibasic sodium phosphate in water. Adjust to a pH of  $5.3 \pm 0.1$ , if necessary.

**Solution B:** Dissolve 400 mg of bromocresol purple in 30 mL of water, add 6.3 mL of 0.1 N sodium hydroxide, and dilute with water to 500 mL.

**Phosphate buffer–bromocresol purple solution:** Mix equal volumes of *Solution A*, *Solution B*, and chloroform, shake in a separator, and discard the chloroform. If appreciable color is extracted, repeat with additional portions of chloroform until no color is extracted.

**Standard stock solution:** 0.8 mg/mL of USP Biperiden Hydrochloride RS in methanol

**Standard solution:** 40  $\mu$ g/mL of USP Biperiden Hydrochloride RS, prepared as follows: Transfer a suitable volume of *Standard stock solution* to a suitable volumetric flask, add 25% of the flask volume of water, and dilute with methanol to volume.

**Sample solution:** Nominal concentration of 40  $\mu$ g/mL of biperiden hydrochloride from NLT 20 Tablets, prepared as follows: Transfer a portion of finely powdered Tablets, to obtain the final nominal concentration, to a suitable volumetric flask; add 25% of the volume of water; and heat on a steam bath for 15 min. Cool, and dilute with methanol to volume.

**Blank:** Methanol and water (3:1)

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Transfer 5.0 mL each of the *Standard solution*, the *Sample solution*, and the *Blank* to individual separators, each containing 10.0 mL of *Phosphate buffer–bromocresol purple solution*. Extract the solution in each separator with 20.0 mL of chloroform for 2 min. After the layers have separated, pass each chloroform extract through filter paper (Whatman No. 31 or equivalent) into separate glass-stoppered, 50-mL volumetric flasks. In the same manner, extract the solution in each separator with another 20.0-mL portion of chloroform, filter, and wash each filter with 8 mL of chloroform, collecting each combined filtrate and washing, respectively, in the 50-mL volumetric flask containing the first extract. Dilute each with chloroform to volume. Concomitantly determine the absorbances of the solutions in 1-cm cells at the wavelength of maximum absorbance at about 408 nm, with a suitable spectrophotometer, using the *Blank* to set the instrument.



Calculate the percentage of the label claim of  $C_{21}H_{29}NO \cdot HCl$  in the Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of USP Biperiden Hydrochloride RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of the *Sample solution* ( $\mu\text{g/mL}$ )  
 Acceptance criteria: 93.0%–107.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 45 min

[NOTE—Determine the amount of  $C_{21}H_{29}NO \cdot HCl$  dissolved by using the following method.]

**Phosphate buffer–bromocresol purple solution:** Prepare as directed in the *Assay*.

**Standard stock solution:** 0.8 mg/mL of USP Biperiden Hydrochloride RS in methanol

**Standard solution:** 2  $\mu\text{g/mL}$  of USP Biperiden Hydrochloride RS, prepared as follows: Pipet 5 mL of *Standard stock solution* into a 500-mL volumetric flask, and add 0.01 N hydrochloric acid to volume. Pipet 25 mL of this solution into a suitable beaker, and adjust with 0.01 N sodium hydroxide to a pH of 5.3. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.

**Sample solution:** Sample per *Dissolution* (711). Filter 75 mL of the solution under test, pipet 50 mL of the clear filtrate into a suitable beaker, and adjust with 0.01 N sodium hydroxide to a pH of 5.3. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.

**Blank:** Water

### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Pipet 20.0 mL each of the *Standard solution*, the *Sample solution*, and the *Blank* into individual separators, each containing 10.0 mL of *Phosphate buffer–bromocresol purple solution*. Extract the solution in each separator with 40.0 mL of chloroform for 10 min. After the layers have separated, pass each chloroform extract through filter paper into separate, glass-stoppered containers, discarding the first 10 mL of each filtrate. Determine the amount of  $C_{21}H_{29}NO \cdot HCl$  dissolved from absorbances at the wavelength of maximum absorbance at about 408 nm (10-cm cells) of the extract from the *Sample solution* in comparison with that of the extract from the *Standard solution*, using the *Blank* to set the instrument.

**Tolerances:** NLT 75% (Q) of  $C_{21}H_{29}NO \cdot HCl$  is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Biperiden Hydrochloride RS

▲ USP40

Delete the following:

## Biperiden Lactate Injection

### DEFINITION

Biperiden Lactate Injection is a sterile solution of biperiden lactate ( $C_{21}H_{29}NO \cdot C_3H_5O_3$ ) in Water for Injection, prepared from Biperiden with the aid of Lactic Acid. It contains NLT 95.0% and NMT 105.0% of the labeled amount of biperiden lactate ( $C_{21}H_{29}NO \cdot C_3H_5O_3$ ).

### IDENTIFICATION

#### • A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181)

**Standard solution:** 50 mg of USP Biperiden RS in 25 mL of 0.01 N hydrochloric acid

**Sample solution:** Nominally 50 mg of biperiden lactate from Injection

**Analysis:** Proceed as directed in *Identification—Organic Nitrogenous Bases* (181), beginning with "Transfer the liquid to a separator".

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Solution A:** 38 g/L of monobasic sodium phosphate and 2 g/L of anhydrous dibasic sodium phosphate in water. Adjust to a pH of  $5.3 \pm 0.1$ , if necessary.

**Solution B:** Dissolve 400 mg of bromocresol purple in 30 mL of water. Add 6.3 mL of 0.1 N sodium hydroxide, and dilute with water to 500 mL.

**Phosphate buffer–bromocresol purple solution:** Mix equal volumes of *Solution A*, *Solution B*, and chloroform. Shake in a separator, and discard the chloroform. If appreciable color is extracted, repeat with additional portions of chloroform until no color is extracted.

**Standard stock solution:** 0.8 mg/mL of USP Biperiden RS in methanol

**Standard solution:** 40  $\mu\text{g/mL}$  of USP Biperiden RS from *Standard stock solution* in solution prepared as follows. Transfer a suitable quantity of *Standard stock solution* to an appropriate volumetric flask. Add 25% of the flask volume of water. Dilute with methanol to volume.

**Sample solution:** Nominally 50  $\mu\text{g/mL}$  of biperiden lactate from Injection in solution prepared as follows. Transfer a suitable quantity of the Injection to an appropriate volumetric flask. Add 25% of the flask volume of water. Dilute with methanol to volume.

**Blank:** Methanol and water (3:1)

### Instrumental conditions

**Mode:** UV-Vis

**Analytical wavelength:** Maximum absorbance at about 408 nm

**Cell:** 1 cm

### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Transfer 5.0 mL each of the *Standard solution*, the *Sample solution*, and the *Blank* to individual separators, each containing 10.0 mL of *Phosphate buffer–bromocresol purple solution*. Extract the solution in each separator with 20.0 mL of chloroform for 2 min. After the layers have separated, pass each chloroform extract through filter paper (Whatman No. 31 or equivalent) into separate glass-stoppered, 50-mL volumetric flasks. In the same manner, extract the solution in each separator with another 20.0-mL portion of chloroform, filter, and wash each filter with 8 mL of chloroform, collecting each combined filtrate and washing, respectively, in the 50-mL volumetric flask containing the first extract. Dilute each with chloroform to volume.

Concomitantly determine the absorbances of the solutions using the *Blank* to set the instrument.



Calculate the percentage of the labeled amount of biperiden lactate ( $C_{21}H_{29}NO \cdot C_3H_6O_3$ ) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

- $A_U$  = absorbance from the *Sample solution*  
 $A_S$  = absorbance from the *Standard solution*  
 $C_S$  = concentration of USP Biperiden RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of biperiden lactate in the *Sample solution* ( $\text{mg/mL}$ )  
 $M_{r1}$  = molecular weight of biperiden lactate, 401.54  
 $M_{r2}$  = molecular weight of biperiden, 311.46  
 Acceptance criteria: 95.0%–105.0%

#### SPECIFIC TESTS

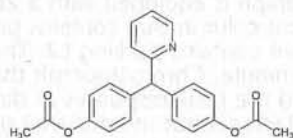
- **PH (791):** 4.8–5.8
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 83.3 USP Endotoxin Units/mg of biperiden lactate
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products (1)*

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, protected from light.
- **USP REFERENCE STANDARDS (11)**  
 USP Biperiden RS  
 USP Endotoxin RS

▲USP40

## Bisacodyl



$C_{22}H_{19}NO_4$  361.39  
 Phenol, 4,4'-(2-pyridinylmethylene)bis-, diacetate (ester);  
 4,4'-(2-Pyridylmethylene)diphenyl diacetate (ester);  
 4,4'-(Pyridin-2-ylmethylene)diphenyl diacetate [603-50-9].

#### DEFINITION

Bisacodyl contains NLT 98.0% and NMT 101.0% of  $C_{22}H_{19}NO_4$ , calculated on the dried basis. [**CAUTION**—Avoid inhalation and contact with the eyes, skin, and mucous membranes.]

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197S)**  
 Cell: 1.0 mm  
 Sample solution: 5 mg/mL in chloroform, previously dried
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard stock solution*, as obtained under *Organic Impurities*.

#### ASSAY

##### PROCEDURE

**Sample solution:** Dissolve 300 mg of Bisacodyl in 60 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any

necessary correction (see *Titrimetry (541)*). Each mL of 0.1 N perchloric acid is equivalent to 36.14 mg of  $C_{22}H_{19}NO_4$ .

**Acceptance criteria:** 98.0%–101.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

#### Delete the following:

- **HEAVY METALS (231), Method II:** NMT 10 ppm (Official 1, Jan-2018)

#### ORGANIC IMPURITIES

**Buffer:** 1.58 g/L of ammonium formate in water, adjusted with formic acid to a pH of 5.0

**Mobile phase:** Acetonitrile and *Buffer* (45:55)

**Diluent:** Acetonitrile and water (35:5)

**Standard stock solution:** 1.0 mg/mL of USP Bisacodyl RS in *Diluent*

**Standard solution:** 1.0  $\mu\text{g/mL}$  of USP Bisacodyl RS in *Diluent*

**System suitability solution:** 0.8 mg/mL of USP Bisacodyl RS; 2  $\mu\text{g/mL}$  each of USP Bisacodyl Related Compounds A, C, and E RS; and 4  $\mu\text{g/mL}$  of USP Bisacodyl Related Compound B RS in *Diluent*

**Sample solution:** 1.0 mg/mL of Bisacodyl in *Diluent*

**Chromatographic system**  
 (See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 4.6-mm  $\times$  25-cm; 4- $\mu\text{m}$  or 5- $\mu\text{m}$  packing L1

**Flow rate:** 1.5 mL/min

**Run time:** 3.5 times the retention time of bisacodyl

**Injection size:** 20  $\mu\text{L}$

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*. [NOTE—See *Table 1* for the relative retention times.]

#### Suitability requirements

**Relative standard deviation:** NMT 5.0% for the bisacodyl peak, *Standard solution*

**Tailing factor:** NMT 2.0 for the bisacodyl peak, *System suitability solution*

**Resolution:** NLT 1.5 between the bisacodyl related compound E and bisacodyl peaks, *System suitability solution*

#### Analysis

**Samples:** *Standard stock solution*, *Standard solution*, and *Sample solution*

[NOTE—Chromatograph the *Standard stock solution* to perform *Identification test B*.]

Calculate the percentage of any individual impurity in the portion of Bisacodyl taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of bisacodyl from the *Standard solution*

$C_S$  = concentration of the *Standard solution* ( $\text{mg/mL}$ )

$C_U$  = concentration of the *Sample solution* ( $\text{mg/mL}$ )

$F$  = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*. [NOTE—The reporting level for impurities is 0.05%.]



Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bisacodyl related compound A <sup>a</sup>	0.20	1.7	0.15
Bisacodyl related compound B <sup>b</sup>	0.40	1.5	0.15
Bisacodyl related compound C <sup>c</sup>	0.45	1.3	0.50
Specified unidentified impurity 1	0.85	1.0	0.20
Bisacodyl related compound E <sup>d</sup>	0.90	1.0	0.50
Bisacodyl	1.0	—	—
Specified unidentified impurity 2	2.6	1.0	0.30
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

<sup>a</sup> 4,4'-Diphenol impurity.<sup>b</sup> 2,4'-Diphenol impurity.<sup>c</sup> Monoacetyl bisacodyl.<sup>d</sup> 2,4'-Bisacodyl analog.**SPECIFIC TESTS**

- **LOSS ON DRYING** (731): Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Bisacodyl RS

USP Bisacodyl Related Compound A RS

4,4'-(Pyridin-2-ylmethylene)diphenol.

C<sub>18</sub>H<sub>15</sub>NO<sub>2</sub> 277.32

USP Bisacodyl Related Compound B RS

2,4'-(Pyridin-2-ylmethylene)diphenol.

C<sub>18</sub>H<sub>15</sub>NO<sub>2</sub> 277.32

USP Bisacodyl Related Compound C RS

4-[(4-Hydroxyphenyl)(pyridin-2-yl)methyl]phenyl acetate.

C<sub>20</sub>H<sub>17</sub>NO<sub>3</sub> 319.35

USP Bisacodyl Related Compound E RS

2-[(4-Acetoxyphenyl)(pyridin-2-yl)methyl]phenyl acetate.

C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub> 361.39**Bisacodyl Suppositories**

» Bisacodyl Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub>.

**Packaging and storage**—Preserve in well-closed containers at a temperature not exceeding 30°.

**USP Reference standards** (11)—

USP Bisacodyl RS

**Identification**—

**A:** Transfer a quantity of Suppositories, equivalent to about 150 mg of bisacodyl, to a 500-mL conical flask, add 75 mL of solvent hexane, and heat on a steam bath until they are melted. Filter the solution, with the aid of vacuum, through a medium-porosity, sintered-glass funnel, and wash the residue with about 100 mL of warm solvent hexane until it is free from fat. Continue the vacuum until the residue

appears dry. Dissolve the residue by rinsing the filter with about 50 mL of warm acetone, collecting the filtrate in a 150-mL beaker, and evaporate the filtrate on a steam bath to a volume of about 5 mL. To the residual liquid add about 75 mL of water, heat on a steam bath for 15 minutes, and cool. Scratch the sides of the beaker to induce crystallization, filter the crystals, and dry at 100° for about 15 minutes: the bisacodyl so obtained melts between 129° and 135°, and responds to *Identification* test A under *Bisacodyl*.

**B:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for bisacodyl, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of 0.074 M sodium acetate in water [adjusted with 2.5% (v/v) acetic acid to a pH of 7.4] and acetonitrile (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bisacodyl RS in acetonitrile to obtain a *Standard preparation* having a known concentration of about 0.5 mg per mL.

**Assay preparation**—Transfer a number of Suppositories, equivalent to about 100 mg of bisacodyl, to a 500-mL separator, add 150 mL of *n*-hexane, and shake until all the suppositories are dissolved. Add 50 mL of acetonitrile, shake for 1 minute, and allow the layers to separate. Drain the lower layer into a 200-mL volumetric flask, and extract the *n*-hexane layer remaining in the separator with two 50-mL portions of acetonitrile, combining the lower layers in the volumetric flask. Dilute the combined extracts in the volumetric flask with acetonitrile to volume, mix, and filter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 265-nm detector, a 3.9-mm × 30-cm column that contains packing L1, and a guard column that contains packing L2. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub> in the Suppositories taken by the formula:

$$200C(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Bisacodyl RS in the *Standard preparation*; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Bisacodyl Rectal Suspension**

» Bisacodyl Rectal Suspension is a suspension of Bisacodyl in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub>.

**Packaging and storage**—Preserve in unit-dose containers at a temperature not exceeding 30°.

**USP Reference standards** (11)—

USP Bisacodyl RS



**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* as obtained in the *Assay*.

**pH** (791): between 5.0 and 6.8.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and 0.01 M monobasic potassium phosphate (60:40). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Dissolve a suitable quantity of ethylparaben in methanol, and dilute with an equal volume of water to obtain a solution containing about 5.0 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bisacodyl RS in methanol, add an accurately measured volume of *Internal standard solution*, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having known concentrations of about 67 µg per mL and 250 µg per mL for bisacodyl and ethylparaben, respectively.

**Assay preparation**—Transfer an accurately measured volume of Rectal Suspension, equivalent to 6.7 mg of bisacodyl, to a 100-mL volumetric flask. Add 5.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 2.0 for bisacodyl and 1.0 for ethylparaben; the resolution, *R*, between bisacodyl and the internal standard is not less than 7.0; the column efficiency, determined for the analyte peak, is not less than 2000 theoretical plates; the tailing factor is not more than 1.2; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub> in the portion of Rectal Suspension taken by the formula:

$$100C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Bisacodyl RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of the bisacodyl peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bisacodyl Delayed-Release Tablets

### DEFINITION

Bisacodyl Delayed-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bisacodyl (C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub>).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197S)

Cell: 1.0 mm

**Sample solution**: Macerate a portion of powdered Tablets, equivalent to 300 mg of bisacodyl, with 100 mL of acetone. Heat on a steam bath to boiling, filter, and evaporate to about 20 mL. Add 200 mL of water, and

warm the mixture on the steam bath, passing a stream of nitrogen over the surface to evaporate the acetone. After 30 min, cool the mixture, and filter through a sintered-glass funnel. Discard the filtrate, and dissolve the crystals in 50 mL of acetone. Evaporate the solution to about 15 mL, add about 75 mL of water, heat on a steam bath for 15 min, and then cool. Scratch the sides of the beaker to induce crystallization, filter the crystals, and dry at 100° for about 15 min. Using the crystals, prepare a solution (1 in 200) in chloroform.

**Acceptance criteria**: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer**: 0.074 M sodium acetate in water, adjusted with 2.5% (v/v) acetic acid to a pH of 7.4

**Mobile phase**: Acetonitrile and *Buffer* (45:55)

**Standard solution**: 0.5 mg/mL of USP Bisacodyl RS in acetonitrile

**Sample solution**: Transfer a portion of finely powdered Tablets equivalent to 100 mg of bisacodyl, to a 200-mL volumetric flask, add 25 mL of water, and shake by mechanical means for 15 min followed by sonication for 15 min. Add 100 mL of acetonitrile, and shake by mechanical means for 15 min followed by sonication for 15 min. Dilute with acetonitrile to volume, mix, and filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode**: LC

**Detector**: UV 265 nm

#### Columns

**Guard**: Packing L2

**Analytical**: 3.9-mm × 30-cm; packing L1

**Flow rate**: 2 mL/min

**Injection volume**: 10 µL

#### System suitability

**Sample**: *Standard solution*

**Suitability requirements**

**Tailing factor**: NMT 2.0

**Relative standard deviation**: NMT 2.0%

#### Analysis

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bisacodyl (C<sub>22</sub>H<sub>19</sub>NO<sub>4</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Bisacodyl RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of bisacodyl in the *Sample solution* (mg/mL)

**Acceptance criteria**: 90.0%–110.0%

### PERFORMANCE TESTS

- **DISINTEGRATION (701)**: Proceed as directed for *Delayed-Release (Enteric-Coated) Tablets*. The Tablets do not disintegrate after 1 h of agitation in simulated gastric fluid TS, but then disintegrate within 45 min in simulated intestinal fluid TS.
- **UNIFORMITY OF DOSAGE UNITS (905)**: Meet the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in well-closed containers at a temperature not exceeding 30°.



• **USP REFERENCE STANDARDS** (11)  
USP Bisacodyl RS

## Milk of Bismuth

» Milk of Bismuth contains bismuth hydroxide and Bismuth Subcarbonate in suspension in water, and yields not less than 5.2 percent and not more than 5.8 percent (w/w) of bismuth trioxide ( $\text{Bi}_2\text{O}_3$ ).

Bismuth Subnitrate .....	80 g
Nitric Acid .....	120 mL
Ammonium Carbonate .....	10 g
Strong Ammonia Solution,	
Purified Water, each, a sufficient	
quantity, to make .....	1000 mL

Mix the Bismuth Subnitrate with 60 mL of Purified Water and 60 mL of the Nitric Acid in a suitable container, and agitate, warming gently until solution is effected. Pour this solution, with constant stirring, into 5000 mL of Purified Water containing 60 mL of the Nitric Acid. Dilute 160 mL of Strong Ammonia Solution with 4300 mL of Purified Water in a glazed or glass vessel of at least 12,000-mL capacity. Dissolve the Ammonium Carbonate in this solution, and then pour the bismuth solution quickly into it with constant stirring. Add sufficient 6 N ammonium hydroxide, if necessary, to render the mixture distinctly alkaline, allow to stand until the precipitate has settled, then pour or siphon off the supernatant, and wash the precipitate twice with Purified Water, by decantation. Transfer the magma to a strainer of close texture, so as to provide continuous washing with Purified Water, the outlet tube being elevated to prevent the surface of the magma from becoming dry. When the washings no longer yield a pink color with phenolphthalein TS, drain the moist preparation, transfer to a graduated vessel, add sufficient Purified Water to make 1000 mL, and mix.

NOTE—This method of preparation may be varied, provided the product meets the following requirements.

**Packaging and storage**—Preserve in tight containers, and protect from freezing.

### Identification—

**A:** It responds to the tests for *Bismuth* (191) and for *Carbonate* (191).

**B:** Add 1 mL of 3 N hydrochloric acid to 1 mL of Milk of Bismuth; a clear solution is produced. Pour the clear solution into 10 volumes of water: a white precipitate is formed.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—The total bacterial count does not exceed 100 cfu per mL and the test for *Escherichia coli* is negative.

**Water-soluble substances**—Boil 10 mL with 90 mL of water for 10 minutes, cool, add water to make the total

volume 100 mL, mix, and filter. Evaporate 50 mL of the filtrate to dryness, and ignite it gently: the weight of the residue does not exceed 5 mg (0.1%).

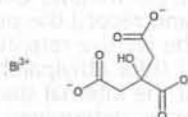
**Arsenic, Method I** (211)—Evaporate 3.75 mL on a steam bath to dryness, add 2 mL of sulfuric acid, and heat until copious fumes of sulfur trioxide are evolved. The limit is 0.8 ppm.

**Lead**—To 5 mL add warm nitric acid, dropwise, until it is just dissolved, and pour the solution into 50 mL of water: a white precipitate may form. Filter, if necessary, evaporate the filtrate on a steam bath to 15 mL, again filter, and to 10 mL of the filtrate add an equal volume of 2 N sulfuric acid: no precipitate is formed.

**Limit of alkalies and alkaline earths**—Dissolve 2.0 mL in 5 mL of hydrochloric acid, dilute with water to 100 mL, add hydrogen sulfide to precipitate the bismuth completely, and filter. To 50 mL of the clear filtrate add 5 drops of sulfuric acid, evaporate to dryness, and ignite: the weight of the residue does not exceed 3 mg (0.3%).

**Assay**—Evaporate an accurately weighed quantity of Milk of Bismuth to dryness, and ignite the residue to constant weight. From the weight of the  $\text{Bi}_2\text{O}_3$  so obtained determine the percentage in the assay specimen.

## Bismuth Citrate



$\text{BiC}_6\text{H}_5\text{O}_7$  398.08 [813-93-4].

» Bismuth Citrate contains not less than 49 percent and not more than 54 percent of bismuth (Bi).

**Packaging and storage**—Preserve in tight, light-resistant containers, store at controlled room temperature, and prevent exposure to excessive heat.

### USP Reference standards (11)—

USP Bismuth Citrate RS

### Identification—

**A:** *Infrared Absorption* (197K): on the undried specimen.

**B:** When strongly heated, the salt chars, and on ignition leaves a more or less blackened residue having a yellow surface. The residue is soluble in warm nitric acid, and this solution, when dropped into a large excess of water, produces a white turbidity.

**C:** Dissolve 1 g in ammonia TS. When treated with hydrogen sulfide in excess, a black precipitate is obtained. Filter this mixture, drive off the excess hydrogen sulfide by heating, and allow to cool. To a portion of this cooled solution add an excess of calcium hydroxide TS, and boil: a white precipitate is formed. Reserve a second portion of the cooled solution for the test for *Limit of nitrate*.

**Arsenic, Method I** (211)—Prepare the *Test Preparation* as follows. Triturate 300 mg with an equal weight of calcium hydroxide, and ignite. Dissolve the residue in 5 mL of 3 N hydrochloric acid: the limit is 10 µg per g.

**Limit of nitrate**—To the second portion of cooled solution reserved from *Identification* test C, add an equal volume of sulfuric acid, mix, and allow to cool. Into the liquid, drop a crystal of ferrous sulfate, and allow to stand for 30 minutes: no brown or brownish black color appears around the crystal.



**Limit of copper, lead, and silver—**

**Standard solution**—Prepare a solution containing 1000 µg of copper per mL, a solution containing 1000 µg of lead per mL, and a solution containing 1000 µg of silver per mL. Transfer 3.0 mL of each solution to a 2000-mL volumetric flask, dilute with 1 N nitric acid to volume, and mix. [NOTE—The concentrations of copper, lead, and silver in this solution may be modified by using a different quantity or by further dilution to bring the absorption responses within the working range of the atomic absorption spectrophotometer.]

**Test solution**—Ignite about 3 g of Bismuth Citrate, accurately weighed, in a porcelain crucible, cool, and cautiously add 6 N nitric acid to dissolve the residue. Add 100 mL of water, and mix. A white precipitate forms. Filter this mixture, evaporate on a steam bath to obtain about 15 mL of solution, and filter again. Dilute the filtrate with water to 20.0 mL.

**Procedure**—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the emission lines of 324.7 nm, 217 nm, and 328.1 nm for copper, lead, and silver, respectively, with an atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with copper, lead, and silver hollow-cathode lamps and an oxidizing flame. The absorbances of the *Test solution* do not exceed those of the *Standard solution* for each element (10 µg per g).

**Limit of soluble bismuth—**

**Standard solution**—Transfer 242.0 mg of bismuth nitrate pentahydrate to a 100-mL volumetric flask. Add 3 mL of 1.5 N nitric acid, swirl to dissolve, dilute with water to volume, and mix. Transfer 1.0 mL of this solution to a 500-mL volumetric flask, add 250 mL of 1.5 N nitric acid, dilute with water to volume, and mix. This solution contains 2.0 µg of bismuth (Bi) per mL. [NOTE—The concentration of bismuth in this solution may be modified by using a different quantity or by further dilution to bring the absorption responses within the working range of the atomic absorption spectrophotometer.]

**Test solution**—Prepare a mixture of 5.0 g of Bismuth Citrate and 100 mL of water, and stir by mechanical means the suspension thus obtained for 2 hours. Pass through filter paper. Pass the filtrate thus obtained through a filter having a 0.1-µm or finer porosity. To 10.0 mL of the filtrate add 0.1 mL of nitric acid.

**Procedure**—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the emission line of 223.06 nm for bismuth with an atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a bismuth hollow-cathode lamp and an oxidizing flame. The absorbances of the *Test solution* do not exceed those of the *Standard solution* (40 µg per g).

**Assay**—Transfer about 300 mg of Bismuth Citrate, accurately weighed, to a porcelain crucible, and ignite. Allow to cool, add 2 mL of nitric acid to the residue, dropwise, and warm until complete solution has been effected. Add about 60 mL of water and 0.3 mL of xylene orange TS, and titrate with 0.05 N edetate disodium VS to a yellow endpoint. Each mL of 0.05 N edetate disodium is equivalent to 10.45 mg of bismuth (Bi).

**Bismuth Subcarbonate****DEFINITION**

Bismuth Subcarbonate contains NLT 97.6% and NMT 100.7% of bismuth subcarbonate  $[(\text{BiO})_2\text{CO}_3]$ , calculated on the dried basis.

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL**, *Bismuth and Carbonate* (191)

**ASSAY**• **PROCEDURE**

**Sample solution**: 500 mg of Bismuth Subcarbonate in 3 mL of nitric acid. Dilute with water to 250 mL, and add 0.3 mL of xylene orange TS.

**Titrimetric system**

**Mode**: Direct titration

**Titrant**: 0.05 M edetate disodium VS

**Endpoint detection**: Visual

**Analysis**: Titrate with *Titrant* to a yellow endpoint. Each mL of 0.05 M edetate disodium is equivalent to 12.75 mg of bismuth subcarbonate  $[(\text{BiO})_2\text{CO}_3]$ .

**Acceptance criteria**: 97.6%–100.7% on the dried basis

**IMPURITIES**

- **CHLORIDE AND SULFATE**, *Chloride* (221)

**Sample stock solution**: 5.0 g in 10 mL of water. Add 20 mL of nitric acid, warm to achieve dissolution, and allow to cool. Dilute with water to obtain 100 mL of solution.

**Sample solution**: To 6.6 mL of the *Sample stock solution* add 4 mL of nitric acid, and dilute with water to 50 mL.

**Acceptance criteria**: A 15.0-mL portion of the *Sample solution* shows no more chloride than corresponds to 70 µL of 0.020 N hydrochloric acid (0.05%).

- **LIMIT OF ALKALIES AND ALKALINE EARTHS**

**Sample solution**: Boil 1.0 g with 20 mL of a mixture of acetic acid and water (1:1). After 2 min, cool, and filter.

**Analysis**: Collect the filtrate, wash the residue with 20 mL of water, and add the washing to the filtrate. To this solution add 2 mL of 2 N hydrochloric acid and 20 mL of water. Heat to boiling, and precipitate the bismuth by adding hydrogen sulfide. Cool the mixture, and filter. Collect the filtrate, wash the residue with water, and add the washing to the filtrate. Evaporate this solution to dryness on a water bath. To the residue add 0.5 mL of sulfuric acid, dry slowly, and cool.

**Acceptance criteria**: The weight of the residue does not exceed 10 mg (1.0%).

- **LIMIT OF NITRATE**

**Indigo carmine titrant**: To 4 g of indigo carmine in 900 mL of water add 2 mL of sulfuric acid, and dilute with water to 1000 mL.

**Standard solution**: 0.0815 mg/mL of potassium nitrate (equivalent to 0.05 mg/mL of nitrate) in water. Place 20.0 mL in a 125-mL conical flask.

**Sample solution**: To 250 mg of Bismuth Subcarbonate in a 125-mL conical flask add 20 mL of water, and swirl to suspend.

**Analysis**: To the *Standard solution* and the *Sample solution* add 0.05 mL of *Indigo carmine titrant*. Carefully add 30 mL of sulfuric acid, and immediately titrate with *Indigo carmine titrant* to a stable blue endpoint.

**Acceptance criteria**: The volume of *Indigo carmine titrant* consumed by the *Sample solution* does not exceed that consumed by the *Standard solution* (0.4%).

- **LIMIT OF SILVER**

**Standard solution**: 7.87 µg/mL of silver nitrate

**Sample solution**: To 2.0 g of Bismuth Subcarbonate add 1 mL of water and 4 mL of nitric acid.

**Analysis**: Heat the *Sample solution* gently to achieve dissolution, add water to obtain 11 mL of solution, and cool. Add 2 mL of 1 N hydrochloric acid, and allow to stand in a dark place for 5 min. Treat the *Standard solution* concomitantly with 1 mL of nitric acid and 2 mL of 1 N hydrochloric acid.

**Acceptance criteria**: The turbidity produced from the *Sample solution* is NMT that produced from the *Standard solution* (0.0025%).



• **LIMIT OF ARSENIC, Method I (211)**

Test preparation: 600 mg in 35 mL of 3 N hydrochloric acid

Acceptance criteria: NMT 5 ppm

• **LIMIT OF COPPER**

Standard stock solution 1: 5 mg/mL of copper prepared as follows. To a 100-mL volumetric flask add 1.34 g of cupric chloride, 10 g of ammonium chloride, and 3 mL of sodium metabisulfite solution (275 mg/mL), and dilute with water to volume.

Standard stock solution 2: 10 µg/mL of copper in 2 N nitric acid from Standard stock solution 1

Standard solution: Mix 0.25 mL of Standard stock solution 2 and 9.75 mL of water.

Sample solution: To 5 mL of the Sample stock solution retained from the test for Chloride and Sulfate, Chloride add 2 mL of 6 N ammonium hydroxide, dilute with water to 50 mL, mix, and filter.

Analysis: To 10 mL each of the Standard solution and the Sample solution add 1 mL of a solution of sodium diethyldithiocarbamate (1 in 1000).

Acceptance criteria: No more color is obtained from the Sample solution than is obtained from the Standard solution (0.005%).

• **LIMIT OF LEAD**

Diluent: 6 N nitric acid, lead-free

Standard stock solution: 0.1598 mg/mL of lead nitrate in Diluent. This solution contains 100 µg/mL of lead.

Standard solutions: 1.0, 2.0, and 3.0 µg/mL of lead from the Standard stock solution in Diluent

Sample solution: 12.5 g of Bismuth Subcarbonate in 75 mL of Diluent. Heat to boiling for 1 min, cool, and dilute with water to 100 mL.

Analysis: Concomitantly determine the absorbances of the Standard solutions and the Sample solution at the lead emission line of 283.3 nm with an atomic absorption spectrophotometer (see Atomic Absorption Spectroscopy (852)) equipped with a lead hollow-cathode lamp and an air-acetylene flame, using a 1:5 dilution of the Diluent as the blank. Plot the absorbances of the Standard solutions versus concentration, in µg/mL, of lead, and draw the straight line best fitting the three plotted points. From the graph, determine the concentration,  $C$ , in µg/mL, of lead in the Sample solution.

Calculate the percentage of lead (Pb) in the portion of Bismuth Subcarbonate taken:

$$\text{Result} = C/1250$$

$C$  = concentration of lead in the Sample solution (µg/mL)

Acceptance criteria: NMT 0.002%

**SPECIFIC TESTS**

• **LOSS ON DRYING (731)**

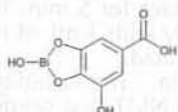
Analysis: Dry at 105° to constant weight.

Acceptance criteria: NMT 1.0% of its weight

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.

## Bismuth Subgallate



$C_7H_5BiO_6$   
Gallic acid bismuth basic salt [99-26-3].

394.09

**DEFINITION**

Bismuth Subgallate is a basic salt that, when dried at 105° for 3 h, contains the equivalent of NLT 52.0% and NMT 57.0% of bismuth trioxide ( $Bi_2O_3$ ).

**IDENTIFICATION**

• **A. IDENTIFICATION TESTS—GENERAL, Bismuth (191)**

Sample: When heated to redness, it at first chars, leaving finally a yellow residue. Use the residue for analysis.

Acceptance criteria: Meets the requirements

• **B.**

Sample: 100 mg

Analysis: Agitate the Sample thoroughly with an excess of hydrogen sulfide TS, filter, and boil the filtrate to expel the dissolved gas. Cool, and add 1 drop of ferric chloride TS.

Acceptance criteria: A purplish blue mixture is produced.

**ASSAY**

• **PROCEDURE**

Sample solution: Dry 1 g of Bismuth Subgallate at 105° for 3 h, then weigh and ignite in a porcelain crucible. Allow it to cool, and add nitric acid to the residue, dropwise, warming until complete solution has been effected.

Analysis: Evaporate the Sample solution to dryness, and carefully ignite the residue to constant weight. From the weight of the residue, determine the percentage of  $Bi_2O_3$  in the portion of Bismuth Subgallate taken.

Acceptance criteria: 52.0%–57.0% on the dried basis

**IMPURITIES**

• **ARSENIC (211)**

Test preparation: 400 mg

Analysis: Triturate the Test preparation with 400 mg of calcium hydroxide, and ignite. Dissolve the residue in 5 mL of 3 N hydrochloric acid.

Acceptance criteria: NMT 7.5 ppm; the solution, without further treatment, meets the requirements.

• **LIMIT OF NITRATE**

Sample: 100 mg

Analysis: Mix the Sample with 5 mL of 2 N sulfuric acid and 5 mL of ferrous sulfate TS, filter the mixture, and carefully superimpose the filtrate, without mixing, on 5 mL of sulfuric acid, in a test tube.

Acceptance criteria: No reddish brown color appears at the zone of contact of the two liquids.

• **LIMITS OF COPPER, LEAD, AND SILVER**

Sample: 3 g

Analysis: Ignite the Sample in a porcelain crucible, cool, and cautiously add, dropwise, just sufficient nitric acid to dissolve the residue upon warming. Evaporate the solution to dryness, again ignite, and cool. Cautiously dissolve the residue in just sufficient nitric acid with the aid of gentle heat, concentrate the solution to about 4 mL, and pour it into 100 mL of water. Filter, evaporate the filtrate on a steam bath to 20 mL, again filter, and divide this filtrate into portions of 5 mL each.

Acceptance criteria

Copper: To 5 mL of the filtrate add a slight excess of 6 N ammonium hydroxide: the liquid does not exhibit a bluish color.

Lead: To 5 mL of the filtrate add 5 mL of 2 N sulfuric acid: the liquid does not become cloudy.

Silver: To 5 mL of the filtrate add hydrochloric acid, dropwise: no precipitate is formed that is insoluble in a slight excess of hydrochloric acid, but that is soluble in 6 N ammonium hydroxide.

• **LIMIT OF ALKALIES AND ALKALINE EARTHS**

Sample: 1.0 g

Analysis: Boil the Sample with 20 mL of a mixture of equal volumes of 6 N acetic acid and water, cool, and filter. Precipitate the bismuth from the filtrate by the addition of hydrogen sulfide, boil the mixture, and fil-



ter. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite to constant weight. Weigh the residue.

Acceptance criteria: NMT 5 mg (0.5%)

• **LIMIT OF FREE GALLIC ACID**

Sample: 1.0 g

Analysis: Shake the *Sample* with 20 mL of alcohol for 1 min, filter, and evaporate the filtrate to dryness on a steam bath, then dry the residue at 105° for 1 h. Weigh the residue.

Acceptance criteria: NMT 5 mg (0.5%)

**SPECIFIC TESTS**

• **LOSS ON DRYING** (731)

Analysis: Dry a sample at 105° for 3 h.

Acceptance criteria: NMT 7.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

## Bismuth Subnitrate

$\text{Bi}_5\text{O}(\text{OH})_9(\text{NO}_3)_4$  1461.99

Bismuth hydroxide nitrate oxide  $\text{Bi}_5\text{O}(\text{OH})_9(\text{NO}_3)_4$ .

Bismuth hydroxide nitrate oxide  $\text{Bi}_5\text{O}(\text{OH})_9(\text{NO}_3)_4$  [1304-85-4].

» Bismuth Subnitrate is a basic salt that contains the equivalent of not less than 79.0 percent of bismuth trioxide ( $\text{Bi}_2\text{O}_3$ ), calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**Identification**—It responds to the tests for *Bismuth* (191) and for *Nitrate* (191).

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 3.0% of its weight.

**Carbonate**—Add 3 g to 3 mL of warm nitric acid: no effervescence occurs. Pour the solution into 100 mL of water: a white precipitate forms. Filter, evaporate the filtrate on a steam bath to 30 mL, again filter the liquid, divide the latter filtrate into portions of 5 mL each, and use these several portions in the tests for *Chloride*, *Sulfate*, *Copper*, *Lead*, and *Silver*.

**Chloride** (221)—A 10-mL portion of the test liquid retained in the test for *Carbonate* shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.035%).

**Sulfate** (221)—To a 5-mL portion of the test liquid retained in the test for *Carbonate* add 5 drops of barium nitrate TS: no turbidity is produced immediately.

**Limit of ammonium salts**—Boil about 100 mg with 5 mL of 1 N sodium hydroxide: the vapor does not turn moistened red litmus paper blue.

**Arsenic, Method I** (211)—Mix 375 mg with 5 mL of water, cautiously add 2 mL of sulfuric acid, and heat the mixture until fumes of sulfur trioxide are copiously evolved. Cool, cautiously add 10 mL of water, and again evaporate to strong fuming, repeating, if necessary, to remove any trace of nitric acid. The limit is 8 ppm.

**Copper**—To a 5-mL portion of the test liquid retained in the test for *Carbonate* add a slight excess of 6 N ammonium hydroxide: the liquid does not exhibit a bluish color.

**Lead**—Mix a 5-mL portion of the test liquid retained in the test for *Carbonate* with an equal volume of 2 N sulfuric acid: the liquid does not become cloudy.

**Silver**—To a 5-mL portion of the test liquid retained in the test for *Carbonate* add hydrochloric acid, dropwise: no pre-

cipitate is formed that is insoluble in a slight excess of hydrochloric acid, but that is soluble in 6 N ammonium hydroxide.

**Limit of alkalis and alkaline earths**—Boil 1.0 g with 20 mL of a mixture of equal volumes of 6 N acetic acid and water, cool, and filter. Add 2 mL of 3 N hydrochloric acid, precipitate the bismuth by the addition of hydrogen sulfide, boil the mixture, and filter it. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite to constant weight: the weight of the residue does not exceed 5 mg (0.5%).

**Assay**—Transfer about 400 mg of Bismuth Subnitrate, accurately weighed, to a 250-mL beaker. Add 5 mL of water, then add 2 mL of nitric acid, and warm, if necessary, to effect solution. Dilute with water to 100 mL, add 0.3 mL of xylene orange TS, and titrate with 0.05 M edetate disodium VS to a yellow endpoint. Each mL of 0.05 M edetate disodium is equivalent to 11.65 mg of  $\text{Bi}_2\text{O}_3$ .

## Bismuth Subsalcylate

$\text{C}_7\text{H}_5\text{BiO}_4$

362.09

(2-Hydroxybenzoato- $\text{O}^1$ )-oxobismuth;

2-Hydroxybenzoic acid bismuth (3+) salt, basic [14882-18-9].

**DEFINITION**

Bismuth Subsalcylate is a basic salt that contains NLT 56.0% and NMT 59.4% of bismuth (Bi) and NLT 36.5% and NMT 39.3% of total salicylates on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197M)

- **B. IDENTIFICATION TESTS—GENERAL**, *Bismuth* (191): Meets the requirements

**ASSAY**

• **BISMUTH**

**Sample solution:** Transfer an equivalent to 300 mg of Bismuth Subsalcylate, previously dried at 105° for 3 h, to a porcelain crucible, and ignite. Allow it to cool, and add about 2 mL of nitric acid to the residue, dropwise, warming until dissolved. Add about 60 mL of water and 0.3 mL of xylene orange TS.

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Titrate the *Sample solution* with *Titrant* to a yellow endpoint. Each mL of *Titrant* is equivalent to 10.45 mg of bismuth (Bi).

**Acceptance criteria:** 56.0%–59.4% of bismuth (Bi) on the dried basis

• **TOTAL SALICYLATES**

**Solution A:** Ferric ammonium sulfate TS, 1 N hydrochloric acid, and water (4:1:15)

**Standard stock solution:** 0.2 mg/mL of USP Salicylic Acid RS in water

**Standard solution:** 0.05 mg/mL of USP Salicylic Acid RS in water, prepared by adding 25.0 mL of *Standard stock solution* and 70 mL of water to a 100-mL volumetric flask. Adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5, before dilution with water to volume.

**Reacted standard solution:** To 25.0 mL of *Standard solution* add 1.0 mL of *Solution A*.

**Unreacted standard solution:** To 25.0 mL of the *Standard solution* add 1.0 mL of 0.05 N hydrochloric acid.

**Sample solution:** Transfer 52 mg of Bismuth Subsalcylate, previously dried at 105° for 3 h, into a 200-mL volumetric flask. Add 10 mL of 0.5 N sodium hydroxide, heat on a steam bath for 15 min, allow to cool, and



dilute with water to volume. Centrifuge 70 mL, and then transfer 50.0 mL of the clear supernatant to a beaker. Add about 40 mL of water, and adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.

**Reacted sample solution:** To 25.0 mL of *Sample solution* add 1.0 mL of *Solution A*.

**Unreacted sample solution:** To 25.0 mL of the *Sample solution* add 1.0 mL of 0.05 N hydrochloric acid.

**Blank:** Water, adjusted with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5.

**Reacted blank solution:** To 25.0 mL of *Blank* add 1.0 mL of *Solution A*.

**Unreacted blank:** To 25.0 mL of *Blank* add 1.0 mL of 0.05 N hydrochloric acid.

#### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** 525 nm

#### Analysis

**Samples:** *Reacted standard solution*, *Unreacted standard solution*, *Reacted sample solution*, *Unreacted sample solution*, *Reacted blank solution*, and *Unreacted blank solution*. Concomitantly determine the absorbances of the *Samples*.

Calculate the percentage of total salicylates in the portion of dried Bismuth Subsalicylate taken:

$$\text{Result} = [(A_{UR} - A_{UU} - B)/(A_{SR} - A_{SU} - B)] \times (C_S/C_U) \times 100$$

$A_{UR}$  = absorbance of the *Reacted sample solution*

$A_{UU}$  = absorbance of the *Unreacted sample solution*

$B$  = difference in the absorption of the *Reacted blank solution* and the absorption of the *Unreacted blank*

$A_{SR}$  = absorbance of the *Reacted standard solution*

$A_{SU}$  = absorbance of the *Unreacted standard solution*

$C_S$  = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Bismuth Subsalicylate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 36.5%–39.3% of total salicylates on the dried basis

#### IMPURITIES

##### • ARSENIC, Method I (211)

**Sample:** 300 mg of Bismuth Subsalicylate with 300 mg of calcium hydroxide

**Analysis:** Triturate the *Sample*, and ignite. Dissolve the residue in 5 mL of 3 N hydrochloric acid.

**Acceptance criteria:** 10 ppm

##### • LIMIT OF COPPER, LEAD, AND SILVER

**Standard stock solution:** Add 3.0 mL each of 1000-μg/mL solutions of copper, lead, and silver, respectively, to a 2000-mL flask, and dilute with 1 M nitric acid to volume.

**Standard solution:** 1.5 μg/mL of copper, 1.5 μg/mL of lead, and 1.5 μg/mL of silver, in 1 M nitric acid from the *Standard stock solution*. The concentrations of copper, lead, and silver may be modified by using different volumes or concentrations to bring the absorption response within the working range of the atomic absorption spectrophotometer.

**Sample solution:** Ignite 3 g of sample in a porcelain crucible, cool, and cautiously add 6 M nitric acid to dissolve the residue, and evaporate on a steam bath. Ignite the residue, cool, transfer the residue to a tared conical flask, and wash the flask with about 5 mL of 6 M nitric acid, adding the wash to the conical flask. Dissolve the residue with the aid of heat, and add water to obtain a solution weighing 20.0 g. The concentrate of Bismuth Subsalicylate may be modified by using the same proportions used for modifying the *Standard solu-*

*tion*, by using a different quantity, or by further dilution.

#### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 324.7 nm for copper; 217 nm for lead; 328.1 nm for silver

**Lamps:** Copper, lead, and silver hollow-cathode, and oxidizing flames

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** 10 ppm; the absorbances of the *Sample solutions* do not exceed those of the *Standard solutions* for each element.

##### • LIMIT OF SOLUBLE BISMUTH

**Standard solution:** 2 μg/mL of bismuth (Bi), prepared as follows. Add 242.0 mg of bismuth nitrate pentahydrate to a 100-mL volumetric flask, add 3 mL of 1.5 M nitric acid, swirl to dissolve, and dilute with water to volume. Add 1.0 mL of this solution to a 500-mL volumetric flask, add 250 mL of 1.5 M nitric acid, and dilute with water to volume. The concentration of bismuth in this solution may be modified by using a lesser dilution or by further dilution to bring the absorption response within the working range of the atomic absorption spectrophotometer.

**Sample solution:** 5.0 g of Bismuth Subsalicylate in 100 mL of water, and stir the suspension thus obtained for 2 h at 20°–23°. Pass through filter paper. Pass the filtrate thus obtained through a filter of 0.1-μm or less pore size. Add 0.1 mL of nitric acid to 10.0 mL of the filtrate. The concentrate of Bismuth Subsalicylate may be modified by using the same proportions used for modifying the *Standard solution*, by using a different quantity, or by further dilution.

#### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 223.06 nm for bismuth

**Lamp:** Bismuth hollow-cathode and an oxidizing flame

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*.

**Acceptance criteria:** 40 ppm; the absorbances of the *Sample solution* do not exceed those of the *Standard solution*.

##### • LIMIT OF NITRATE

**Standard solution:** To 0.1 g of salicylic acid add 6 mL of water, 4.0 mL of a solution containing 100 μg of nitrate per mL, and 20 mL of sulfuric acid. Prepare concomitantly with the *Sample solution*.

**Sample solution:** Add 10 mL of water to 0.1 g of Bismuth Subsalicylate. Carefully add 20 mL of sulfuric acid.

**Acceptance criteria:** 0.4%; the *Sample solution* should not be more yellow than the *Standard solution*.

##### • LIMIT OF FREE SALICYLIC ACID

**Mobile phase:** Methanol and 0.06 M acetic acid (55:45)

**Diluent:** Acetonitrile and water (1:1)

**Standard solution:** 0.02 mg/mL of USP Salicylic Acid RS in *Diluent*

**Sample solution:** Add 260 mg of Bismuth Subsalicylate to a glass centrifuge tube, add about 12 mL of acetonitrile, shake by mechanical means for 20 min, and centrifuge. Decant the supernatant into a suitable container. Repeat the acetonitrile addition, shaking, centrifuging, and decanting. Combine the decanted liquid with the first decantate. Pass the combined liquid through a filter of 0.5-μm or finer pore size, and collect the filtrate in a 50-mL volumetric flask. Wash the container with 5 mL of acetonitrile, and filter the wash, collecting the filtrate in the volumetric flask. Dilute with water to volume.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 300 nm

Columns

Guard: 3.2-mm × 1.5-cm; 5-μm packing L1

Analytical: 4.6-mm × 30-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

**System suitability**Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of free salicylic acid in the portion of Bismuth Subsalicylate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 $r_u$  = peak area of salicylic acid from the *Sample solution*
 $r_s$  = peak area of salicylic acid from the *Standard solution*
 $C_s$  = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

 $C_u$  = concentration of the Bismuth Subsalicylate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.2%

**SPECIFIC TESTS****• pH (791)**

Sample solution: 10 g of Bismuth Subsalicylate in 90 mL of water

Analysis: Shake by mechanical means for 10 min, and filter.

Acceptance criteria: 2.7–5.0

**• LOSS ON DRYING (731)**

Analysis: Dry at 105° for 3 h.

Acceptance criteria: NMT 1.0%

**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.**• USP REFERENCE STANDARDS (11)**

USP Bismuth Subsalicylate RS

USP Salicylic Acid RS

**Bismuth Subsalicylate Magma****DEFINITION**

Bismuth Subsalicylate Magma is a suspension of Bismuth Subsalicylate in water that contains NLT 90.0% and NMT 110.0% of the labeled amount of bismuth subsalicylate ( $C_7H_5BiO_4$ ). Bismuth subsalicylate is a basic salt that when dried at 105° for 3 h contains NLT 56.0% and NMT 59.4% bismuth (Bi) and NLT 36.5% and NMT 39.3% of total salicylates.

Dry at 105° for 3 h to determine the solids content and, after determining the solids content, perform all tests on a portion of the dried Magma.

**IDENTIFICATION****• A. INFRARED ABSORPTION (197M)****• B. IDENTIFICATION TESTS—GENERAL, Bismuth (191):** Meets the requirements**ASSAY****• BISMUTH**

Sample solution: Transfer an equivalent to 300 mg of bismuth subsalicylate, previously dried at 105° for 3 h,

to a porcelain crucible, and ignite. Allow it to cool, and add about 2 mL of nitric acid to the residue, dropwise, warming until dissolved. Add about 60 mL of water and 0.3 mL of xylenol orange TS.

**Titrimetric system**

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Titrate the *Sample solution* with *Titrant* to a yellow endpoint. Each mL of *Titrant* is equivalent to 10.45 mg of bismuth (Bi).

Acceptance criteria: 56.0%–59.4% of bismuth on the previously dried basis

**• TOTAL SALICYLATES**

**Solution A:** Ferric ammonium sulfate TS, 1 N hydrochloric acid, and water (4:1:15)

**Standard stock solution:** 0.2 mg/mL of USP Salicylic Acid RS in water

**Standard solution:** 0.05 mg/mL of USP Salicylic Acid RS in water, prepared by adding 25.0 mL of *Standard stock solution* and 70 mL of water to a 100-mL volumetric flask. Adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5, before dilution with water to volume.

**Reacted standard solution:** To 25.0 mL of *Standard solution* add 1.0 mL of *Solution A*.

**Unreacted standard solution:** To 25.0 mL of the *Standard solution* add 1.0 mL of 0.05 N hydrochloric acid.

**Sample solution:** Transfer an equivalent to 52 mg of bismuth subsalicylate from previously dried Magma at 105° for 3 h to a 200-mL volumetric flask. Add 10 mL of 0.5 N sodium hydroxide, heat on a steam bath for 15 min, allow to cool, and dilute with water to volume. Centrifuge 70 mL, and then transfer 50.0 mL of the clear supernatant to a beaker. Add about 40 mL of water, and adjust with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5. Transfer this solution to a 100-mL volumetric flask with the aid of water, and dilute with water to volume.

**Reacted sample solution:** To 25.0 mL of *Sample solution* add 1.0 mL of *Solution A*.

**Unreacted sample solution:** To 25.0 mL of the *Sample solution* add 1.0 mL of 0.05 N hydrochloric acid.

**Blank:** Water, adjusted with 0.5 N sodium hydroxide or 1 N hydrochloric acid to a pH of 4.5

**Reacted blank solution:** To 25.0 mL of *Blank* add 1.0 mL of *Solution A*.

**Unreacted blank:** To 25.0 mL of *Blank* add 1.0 mL of 0.05 N hydrochloric acid.

**Instrumental conditions**

Mode: UV

Analytical wavelength: 525 nm

**Analysis**

**Samples:** *Reacted standard solution*, *Unreacted standard solution*, *Reacted sample solution*, *Unreacted sample solution*, *Reacted blank solution*, and *Unreacted blank solution*. Concomitantly determine the absorbances of the *Samples*.

Calculate the percentage of total salicylates in the portion of dried Magma taken:

$$\text{Result} = [(A_{UR} - A_{UU} - B)/(A_{SR} - A_{SU} - B)] \times (C_s/C_u) \times 100$$

 $A_{UR}$  = absorbance of the *Reacted sample solution*
 $A_{UU}$  = absorbance of the *Unreacted sample solution*
 $B$  = difference in the absorption of the *Reacted blank solution* and the absorption of the *Unreacted blank*
 $A_{SR}$  = absorbance of the *Reacted standard solution*
 $A_{SU}$  = absorbance of the *Unreacted standard solution*
 $C_s$  = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

 $C_u$  = concentration of bismuth subsalicylate in the *Sample solution* (mg/mL)



Acceptance criteria: 36.5%–39.3% of total salicylates on the previously dried basis

## IMPURITIES

### • LIMIT OF COPPER, LEAD, AND SILVER

**Standard stock solution:** Add 3.0 mL each of 1000-µg/mL solutions of copper, lead, and silver, respectively, to a 2000-mL flask, and dilute with 1 M nitric acid to volume.

**Standard solution:** 1.5 µg/mL of copper, 1.5 µg/mL of lead, and 1.5 µg/mL of silver, in 1 M nitric acid from the *Standard stock solution*. The concentrations of copper, lead, and silver may be modified by using different volumes or concentrations to bring the absorption response within the working range of the atomic absorption spectrophotometer.

**Sample solution:** Ignite 3 g of sample in a porcelain crucible, cool, cautiously add 6 M nitric acid to dissolve the residue, and evaporate on a steam bath. Ignite the residue, cool, transfer the residue to a tared conical flask, and wash the flask with about 5 mL of 6 M nitric acid, adding the wash to the conical flask. Dissolve the residue with the aid of heat, and add water to obtain a solution weighing 20.0 g. The concentrate of bismuth subsalicylate may be modified by using the same proportions used for modifying the *Standard solution*, by using a different quantity, or by further dilution.

### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 324.7 nm for copper; 217 nm for lead; 328.1 nm for silver

**Lamps:** Copper, lead and silver hollow-cathode, and oxidizing flames

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*

**Acceptance criteria:** 10 ppm; the absorbances of the *Sample solutions* do not exceed those of the *Standard solutions* for each element.

### • LIMIT OF SOLUBLE BISMUTH

**Standard solution:** 2 µg/mL of bismuth (Bi), prepared as follows. Add 242.0 mg of bismuth nitrate pentahydrate to a 100-mL volumetric flask, add 3 mL of 1.5 M nitric acid, swirl to dissolve, and dilute with water to volume. Add 1.0 mL of this solution to a 500-mL volumetric flask, add 250 mL of 1.5 M nitric acid, and dilute with water to volume. The concentration of bismuth in this solution may be modified by using a lesser dilution or by further dilution to bring the absorption response within the working range of the atomic absorption spectrophotometer.

**Sample solution:** 5.0 g of bismuth subsalicylate from dried Magma in 100 mL of water, and stir the suspension thus obtained for 2 h at 20°–23°. Pass through filter paper. Pass the filtrate thus obtained through a filter of 0.1-µm or less pore size. Add 0.1 mL of nitric acid to 10.0 mL of the filtrate. The concentrate of bismuth subsalicylate may be modified by using the same proportions used for modifying the *Standard solution*, by using a different quantity, or by further dilution.

### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 223.06 nm for bismuth

**Lamp:** Bismuth hollow-cathode and an oxidizing flame

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solution* and the *Sample solution*.

**Acceptance criteria:** 40 ppm; the absorbances of the *Sample solution* do not exceed those of the *Standard solution*.

### • LIMIT OF NITRATE

**Standard solution:** To 0.1 g of salicylic acid add 6 mL of water, 4.0 mL of a solution containing 100 µg of nitrate per mL, and 20 mL of sulfuric acid. Prepare concomitantly with the *Sample solution*.

**Sample solution:** Add 10 mL of water to 0.1 g of Magma. Carefully add 20 mL of sulfuric acid, and mix.

**Acceptance criteria:** 0.4%; the *Sample solution* should not be more yellow than the *Standard solution*.

### • LIMIT OF FREE SALICYLIC ACID

**Mobile phase:** Methanol and 0.06 M acetic acid (550:450)

**Diluent:** Acetonitrile and water (1:1)

**Standard solution:** 0.02 mg/mL of USP Salicylic Acid RS in *Diluent*

**Sample solution:** Add 260 mg of bismuth subsalicylate from dried Magma to a glass centrifuge tube, add about 12 mL of acetonitrile, shake by mechanical means for 20 min, and centrifuge. Decant the supernatant into a suitable container. Repeat the acetonitrile addition, shaking, centrifuging, and decanting, combining the decanted liquid with the first decantate. Pass the combined liquid through a filter of 0.5-µm pore size, collecting the filtrate in a 50-mL volumetric flask. Wash the container with 5 mL of acetonitrile, and filter the wash, collecting the filtrate in the volumetric flask. Dilute with water to volume.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

### Columns

**Guard:** 3.2-mm × 1.5-cm; 5-µm packing L1

**Analytical:** 4.6-mm × 30-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of free salicylic acid in the portion of Magma taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of salicylic acid from the *Sample solution*

$r_S$  = peak area of salicylic acid from of the *Standard solution*

$C_S$  = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the bismuth subsalicylate in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.2%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **LABELING:** The label states that this article is not intended for direct administration to humans or animals.

### • USP REFERENCE STANDARDS (11)

USP Bismuth Subsalicylate RS

USP Salicylic Acid RS

## Bismuth Subsalicylate Oral Suspension

### DEFINITION

Bismuth Subsalicylate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of bismuth sub-



salicylate ( $C_7H_5BiO_4$ ). It may contain one or more suitable buffers, coloring agents, flavors, preservatives, stabilizers, sweeteners, and suspending agents.

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL** (191), *Bismuth*: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL** (191), *Salicylate*: Meets the requirements for the response to ferric chloride TS after acidifying with nitric acid

#### ASSAY

##### • PROCEDURE

**Standard stock solution:** 2.5 mg/mL of bismuth in nitric acid. Prepare by dissolving in 6% of the flask volume of nitric acid and diluting with 0.01 N nitric acid to volume.

**Standard solution:** 0.05 mg/mL of bismuth in 1 N nitric acid from the *Standard stock solution*

**Sample solution:** Transfer 10 g of Oral Suspension, previously well shaken in its original container to ensure homogeneity, to a 200-mL volumetric flask. Add about 100 mL of 1 N nitric acid, and dilute with 1 N nitric acid to volume. Mix well without shaking, transfer 10.0 mL of this mixture to a 100-mL volumetric flask, and dilute with 1 N nitric acid to volume. Centrifuge about 20 mL at 4500 rpm for at least 10 min.

##### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 463 nm

**Cell:** 1 cm

**Blank:** 1 N nitric acid

##### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
Transfer a measured volume of the *Sample solution* that contains 0.9 mg of bismuth subsalicylate and 10 mL of the *Standard solution* to separate 50-mL volumetric flasks. Add 10.0 mL of 10% ascorbic acid solution and 25.0 mL of 20% potassium iodide solution to each volumetric flask, and dilute with water to volume. Concomitantly determine the absorbances of both solutions, using the *Blank* to set the spectrophotometer. Calculate the percentage of the labeled amount of bismuth subsalicylate ( $C_7H_5BiO_4$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of bismuth in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bismuth subsalicylate in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of bismuth subsalicylate, 362.09

$M_{r2}$  = molecular weight of bismuth, 208.98

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count is NMT  $10^2$  cfu/g, and the total combined molds and yeasts count is NMT  $5 \times 10^1$  cfu/g. It meets the requirements of the test for the absence of *Escherichia coli*.
- **PH** (791): 3.0–5.5

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from freezing. Avoid excessive heat (over 40°).

## Bismuth Subsalsalicylate Tablets

#### DEFINITION

Bismuth Subsalsalicylate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bismuth subsalsalicylate ( $C_7H_5BiO_4$ ).

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL**, *Bismuth* (191): Meet the requirements
- **B. IDENTIFICATION TESTS—GENERAL**, *Salicylate* (191): After acidifying with nitric acid, it meets the requirements of the test with ferric chloride TS.

#### ASSAY

##### • PROCEDURE

**Standard stock solution:** 2.5 mg/mL of bismuth in nitric acid. Prepare by dissolving in 6% of the flask volume of nitric acid, and diluting with 0.01 N nitric acid to volume.

**Standard solution:** 0.05 mg/mL of bismuth in 1 N nitric acid from the *Standard stock solution*

**Sample stock solution:** Equivalent to 90 mg of bismuth subsalsalicylate from finely powdered Tablets in a 200-mL volumetric flask. Add 150 mL of 1 N nitric acid, and sonicate for 2 min. Dilute with 1 N nitric acid to volume.

**Sample solution:** Transfer 20.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with 1 N nitric acid to volume. Centrifuge a portion at 4500 rpm for at least 10 min.

##### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 463 nm

**Cell:** 1 cm

**Blank:** 10% ascorbic acid solution, 20% potassium iodide solution, and 1 N nitric acid (2:5:1)

##### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Transfer 10.0 mL of the *Standard solution* and the *Sample solution* to separate 50.0-mL volumetric flasks, and dilute with the *Blank* to volume. Concomitantly determine the absorbance of the solutions at the wavelength of maximum absorbance at 463 nm with a suitable spectrophotometer, using the combined reagent solutions as the blank.

Calculate the percentage of the labeled amount of bismuth subsalsalicylate ( $C_7H_5BiO_4$ ) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of bismuth in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of bismuth subsalsalicylate, 362.09

$M_{r2}$  = molecular weight of bismuth, 208.98

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISINTEGRATION (701)

This test does not apply to Tablets labeled as chewable.

**Time:** 10 min

**Acceptance criteria:** Meet the requirements

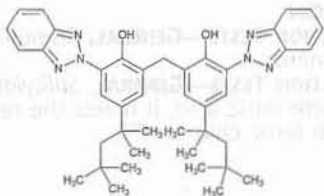
#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Avoid excessive heat (over 40°).



- **LABELING:** Label chewable Tablets to indicate that they are to be chewed before swallowing.

## Bisotrizole



$C_{41}H_{50}N_6O_2$  658.87  
 Phenol, 2,2'-methylenebis[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)]-;  
 2,2'-Methylenebis[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol] [103597-45-1].

### DEFINITION

Bisotrizole contains NLT 96.0% and NMT 102.0% of bisotrizole ( $C_{41}H_{50}N_6O_2$ ), calculated on the as-is basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Diluent:** Tetrahydrofuran and 0.2% (w/v) aqueous solution of 1-pentane sulfonic acid sodium salt (60:40)

**Solution A:** 0.4 g of 1-pentane sulfonic acid sodium salt, 800 mL of methanol, 200 mL of water, and 0.5 mL of phosphoric acid

**Solution B:** 0.4 g of 1-pentane sulfonic acid sodium salt, 1000 mL of methanol, and 0.5 mL of phosphoric acid

**Mobile phase:** See Table 1. Return to original conditions and re-equilibrate the system.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	70	30
1	70	30
11	3	97
40	3	97

**System suitability solution:** 0.8 mg/mL of bisotrizole from USP Bisotrizole Resolution Mixture RS prepared as follows. Transfer USP Bisotrizole Resolution Mixture RS to a suitable volumetric flask, dissolve in tetrahydrofuran, and dilute with *Diluent* to volume.

**Standard solution:** 0.8 mg/mL of USP Bisotrizole RS prepared as follows. Transfer USP Bisotrizole RS to a suitable volumetric flask, dissolve in tetrahydrofuran equivalent to 60% of the final volume, and dilute with *Diluent* to volume.

**Sample solution:** Transfer 80 mg of Bisotrizole to a 100-mL volumetric flask. Dissolve in 60 mL of tetrahydrofuran, and dilute with *Diluent* to volume.

### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 346 nm

**Column:** 3.0-mm × 25-cm; 5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 0.8 mL/min

**Injection volume:** 10 μL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for the relative retention times for bisotrizole and the bisotrizole isomer.]

### Suitability requirements

**Resolution:** NLT 1.5 between bisotrizole and the bisotrizole isomer, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of bisotrizole ( $C_{41}H_{50}N_6O_2$ ) in the portion of Bisotrizole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Bisotrizole RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Bisotrizole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 96.0%–102.0% on the as-is basis

### IMPURITIES

#### Delete the following:

- **HEAVY METALS** (231), *Method II*: NMT 20 ppm (Official 1-Jan-2018)

#### LIMIT OF BISOTRIZOLE RELATED COMPOUND A AND BISOTRIZOLE ISOMER

**Diluent, Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**Standard stock solution A:** 0.65 mg/mL of USP Bisotrizole RS in tetrahydrofuran

**Standard stock solution B:** 0.40 mg/mL of USP Bisotrizole Related Compound A RS in tetrahydrofuran

**Standard solution:** Transfer 5 mL of *Standard stock solution A* and 1.0 mL of *Standard stock solution B* to a 100-mL volumetric flask. Add 60 mL of tetrahydrofuran, and dilute with *Diluent* to volume.

### System suitability

**Sample:** *System suitability solution*

[NOTE—See Table 2 for the relative retention times for bisotrizole related compound A and the bisotrizole isomer.]

### Suitability requirements

**Resolution:** NLT 1.5 between bisotrizole and the bisotrizole isomer

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of bisotrizole related compound A in the portion of Bisotrizole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of bisotrizole related compound A from the *Sample solution*

$r_S$  = peak response of bisotrizole related compound A from the *Standard solution*

$C_S$  = concentration of USP Bisotrizole Related Compound A RS in the *Standard solution* (mg/mL)



$C_U$  = concentration of Bisoprolol in the *Sample solution* (mg/mL)  
Calculate the percentage of bisoprolol isomer in the portion of Bisoprolol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of bisoprolol isomer from the *Sample solution*  
 $r_S$  = peak response of bisoprolol from the *Standard solution*  
 $C_S$  = concentration of USP Bisoprolol RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Bisoprolol in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 2*.

#### • ORGANIC IMPURITIES

Diluent, Solution A, Solution B, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the *Assay*.

#### Analysis

Sample: *Sample solution*

Calculate the percentage of each individual unspecified impurity in the portion of Bisoprolol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each individual impurity  
 $r_T$  = sum of the responses of all the peaks

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bisoprolol related compound A <sup>a</sup>	0.42	0.5
Bisoprolol	1.0	—
Bisoprolol isomer <sup>b</sup>	1.1	4.0
Any individual unspecified impurity	—	0.10
Total impurities	—	4.0

<sup>a</sup> 2-(2H-Benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol.

<sup>b</sup> Phenol, 2,2-methylenebis[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)].

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.

#### • USP REFERENCE STANDARDS (11)

USP Bisoprolol RS

USP Bisoprolol Related Compound A RS

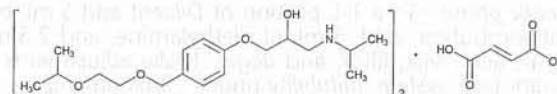
2-(2H-Benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl) phenol.

$C_{20}H_{25}N_3$  323.43

USP Bisoprolol Resolution Mixture RS

A mixture of approximately 1.5% of bisoprolol isomer [phenol, 2,2-methylenebis[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)]] in a matrix of bisoprolol.

## Bisoprolol Fumarate



$(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  766.96

2-Propanol, 1-[4-[[2-(1-methylethoxy)ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino]-, (±)-, (E)-2-butenedioate (2:1) (salt).

(±)-1-[[α-(2-Isopropoxyethoxy)-p-tolyl]oxy]-3-(isopropylamino)-2-propanol fumarate (2:1) (salt) [104344-23-2].

» Bisoprolol Fumarate contains not less than 97.5 percent and not more than 102.0 percent of  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at controlled room temperature.

#### USP Reference standards (11)—

USP Bisoprolol Fumarate RS

#### Identification—

A: *Infrared Absorption* (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Specific rotation** (781): between  $-2^\circ$  and  $+2^\circ$ .

*Test solution:* 10 mg per mL, in methanol.

**Water Determination, Method I** (921): not more than 0.5%.

**Residue on ignition** (281): not more than 0.1%.

#### Delete the following:

• **Heavy metals, Method I** (231): 0.002%. • (Official 1-Jan-2018)

#### Chromatographic purity—

*Diluent, Mobile phase, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay*.

*Standard solution*—Prepare as directed for *Standard preparation* in the *Assay*.

*Test solution*—Prepare as directed for *Assay preparation* in the *Assay*.

*Procedure*—Inject a volume (about 10  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak areas. Calculate the percentage of total impurities in the portion of Bisoprolol Fumarate taken by the formula:

$$100(r_i/r_s)$$

in which  $r_i$  is the sum of areas for all the peaks, excluding the fumaric acid and bisoprolol peaks; and  $r_s$  is the sum of the areas of all the peaks in the chromatogram: not more than 0.5% of total impurities is found.

**Content of fumaric acid**—Transfer about 500 mg of Bisoprolol Fumarate, accurately weighed, to a beaker, and dissolve in 70 mL of dehydrated alcohol. Add 8.0 mL of 0.1 N tetrabutylammonium hydroxide VS, and stir for 2 minutes. Titrate with 0.1 N tetrabutylammonium hydroxide VS, determining the endpoint potentiometrically, using a glass-calomel electrode system. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N tetrabutylammonium hydroxide is equivalent to 5.804 mg of fumaric acid: not less than 14.8% and not more than 15.4% of fumaric acid is found, calculated on the anhydrous basis.



**Assay—**

**Diluent**—Prepare a mixture of water and acetonitrile (65:35).

**Mobile phase**—To a 1-L portion of *Diluent* add 5 mL of heptafluorobutyric acid, 5 mL of diethylamine, and 2.5 mL of formic acid. Mix, filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Prepare a solution in *Diluent* containing about 0.5 mg of propranolol hydrochloride and 1 mg of Bisoprolol Fumarate per mL.

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Bisoprolol Fumarate RS in *Diluent* to obtain a solution having a known concentration of about 1 mg per mL.

**Assay preparation**—Transfer about 50 mg of Bisoprolol Fumarate, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 273-nm detector and a 4.6-mm × 12.5-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak areas as directed for *Procedure*: the resolution, *R*, between bisoprolol and propranolol is not less than 7.0. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  in the portion of Bisoprolol Fumarate taken by the formula:

$$50C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Bisoprolol Fumarate RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bisoprolol Fumarate Tablets

**DEFINITION**

Bisoprolol Fumarate Tablets contain NLT 90.0% and NMT 105.0% of the labeled amount of bisoprolol fumarate  $[(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4]$ .

**IDENTIFICATION**

- THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Sample solution**: Equivalent to 40 mg of bisoprolol fumarate, from powdered Tablets (NLT 5), in a 50-mL flask. Add about 20 mL of a mixture of dichloromethane and methanol (7:3), shake for 30 min, centrifuge, and use the clear solution.

**Application volume**: 20 µL

**Developing solvent system**: Dichloromethane, methanol, and ammonia TS, stronger (70:10:0.8)

**Analysis**

**Sample**: *Sample solution*

Proceed as directed in the chapter, except to develop the chromatogram until the solvent front has moved about two-thirds of the length of the plate and to dry the plate in a current of cold air.

**ASSAY**

- PROCEDURE**

**Diluent**: Acetonitrile and water (7:13)

**Mobile phase**: A 1-L portion of *Diluent*. Add 5 mL of heptafluorobutyric acid, 5 mL of diethylamine, and 2.5 mL of formic acid.

**System suitability solution**: 0.5 mg/mL of propranolol hydrochloride and 1 mg/mL of bisoprolol fumarate in *Diluent*

**Standard solution**: 1 mg/mL of USP Bisoprolol Fumarate RS in *Diluent*

**Sample solution**: Transfer an equivalent of 25 mg of bisoprolol fumarate, from powdered Tablets (NLT 20), to a 25-mL volumetric flask. Add 10 mL of *Diluent*, and sonicate for 10 min. Cool, dilute with *Diluent* to volume, and mix. Centrifuge for 20 min, and use the clear supernatant.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode**: LC

**Detector**: UV 273 nm

**Column**: 4.6-mm × 12.5-cm; packing L7

**Flow rate**: 1 mL/min

**Injection size**: 10 µL

**System suitability**

**Samples**: *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution**: NLT 7.0 between bisoprolol and propranolol, *System suitability solution*

**Tailing factor**: NMT 2.0, *Standard solution*

**Relative standard deviation**: NMT 2.0%, *Standard solution*

**Analysis**

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  in the portion of Tablets taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Bisoprolol Fumarate RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of bisoprolol fumarate in the *Sample solution* (mg/mL)

**Acceptance criteria**: 90.0%–105.0%

**PERFORMANCE TESTS**

- DISSOLUTION (711)**

**Test 1**

**Medium**: Water; 900 mL

**Apparatus 2**: 75 rpm

**Time**: 20 min

Determine the amount of  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  dissolved by using the following method.

**Diluent**: Methanol, triethylamine, phosphoric acid, and water (160:5:2.5:35)

**Mobile phase**: Methanol, triethylamine, and water (34:1:50). Adjust with phosphoric acid to a pH of 4.0 ± 0.1.

**Standard stock solution**: USP Bisoprolol Fumarate RS in water to obtain a solution having a known concentration of about twice the concentration of bisoprolol fumarate in the *Sample solution*

**Standard solution**: *Standard stock solution* and *Diluent* (1:1)

**Sample solution**: *Sample per Dissolution (711)*. Withdraw a portion of the solution under test, filter, and dilute with an equal volume of *Diluent*.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 227 nm**Column:** 4.6-mm × 33-mm; packing L7**Flow rate:** 1 mL/min**Injection size:** 50 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution***Tolerances:** NLT 80% (Q) of the labeled amount of  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.**Medium:** 0.5 M sodium chloride; 900 mL**Apparatus 2:** 75 rpm**Time:** 20 min**Analysis:** Proceed as directed for *Test 1* with the following modifications.**Diluent:** Prepare a mixture of methanol, 0.1 N hydrochloric acid, triethylamine, and phosphoric acid (160: 35: 5: 2.5). The dimensions of the column are 4.6 mm × 25 cm.**Tolerances:** NLT 80% (Q) of the labeled amount of  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)
  - USP Bisoprolol Fumarate RS
  - 2-Propanol, 1-[4-[[2-(1-methylethoxy)ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino]-, (±)-, (E)-2-butenedioate (2:1) (salt).
  - $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  766.96

## Bisoprolol Fumarate and Hydrochlorothiazide Tablets

» Bisoprolol Fumarate and Hydrochlorothiazide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of bisoprolol fumarate  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  and hydrochlorothiazide  $(C_7H_8ClN_3O_4S_2)$ .

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at controlled room temperature.

**USP Reference standards** (11)—

USP Bisoprolol Fumarate RS

USP Chlorothiazide RS

USP Hydrochlorothiazide RS

**Identification**—**A:** *Thin-Layer Chromatographic Identification Test* (201)—

**Test solution**—Finely powder 1 Tablet, and transfer the powder to a 5-mL volumetric flask. Dilute with methanol to volume, sonicate for 5 minutes, centrifuge, and use the supernatant.

**Standard solution 1**—Dissolve a suitable quantity of USP Bisoprolol Fumarate RS in methanol to obtain a solution containing 1 mg per mL.

**Standard solution 2**—Dissolve a suitable quantity of USP Hydrochlorothiazide RS in methanol to obtain a solution containing 1 mg per mL.

**Application volume:** 25 µL.

**Developing solvent system:** a mixture of methylene chloride, methanol, and 14.5 M ammonium hydroxide solution (43:20:8).

**Procedure**—Locate the spots on the plate under short-wavelength UV light and by exposure to iodine vapors: the  $R_f$  values of the principal spots in the chromatogram obtained from the *Test solution* correspond to those of the principal spots in the chromatograms obtained from *Standard solution 1* and *Standard solution 2*.

**B:** The retention times of the major peaks in the chromatograms of the *Bisoprolol fumarate assay preparation* and the *Hydrochlorothiazide assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—**Medium:** 0.1 N hydrochloric acid; 900 mL.**Apparatus 2:** 75 rpm.

**Times:** 20 minutes for bisoprolol fumarate; 30 minutes for hydrochlorothiazide.

**Triethylamine solution**—Mix 2 mL of triethylamine with 1000 mL of water, and adjust with phosphoric acid to a pH of 3.0.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile and *Triethylamine solution* (1:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard stock solution 1**—Quantitatively dissolve an accurately weighed quantity of USP Bisoprolol Fumarate RS in *Medium* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Standard stock solution 2**—Transfer about 30 mg of USP Hydrochlorothiazide RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Medium* to volume, and mix.

**Standard solution**—Dilute accurately measured volumes of *Standard stock solution 1* and *Standard stock solution 2* with *Medium* to obtain a solution having known concentrations of bisoprolol fumarate and hydrochlorothiazide corresponding to those of the solution under test.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a UV detector capable of measuring peak responses at 227 nm and 272 nm, simultaneously, and a 3.9-mm × 15-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the filtered portions of the solution under test into the chromatograph, record the chromatograms, and measure the peak areas for bisoprolol at 227 nm and for hydrochlorothiazide at 272 nm. Calculate the quantities, in mg, of bisoprolol fumarate  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  and hydrochlorothiazide  $(C_7H_8ClN_3O_4S_2)$  dissolved.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $(C_{18}H_{31}NO_4)_2 \cdot C_4H_4O_4$  is dissolved in 20 minutes and not less than 80% (Q) of the labeled amount of  $C_7H_8ClN_3O_4S_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): Meet the requirements with respect to bisoprolol fumarate and to hydrochlorothiazide.

**Chromatographic purity**—

**Diluent, Solution A, Solution B, Mobile phase, and System suitability solution**—Proceed as directed in the *Assay*.

**Standard solution**—Dissolve an accurately weighed quantity of USP Hydrochlorothiazide RS in *Diluent*, and quantita-



tively dilute with *Diluent*, if necessary, to obtain a solution having a known concentration of about 2 µg per mL.

**Test stock solution**—Proceed as directed for *Assay stock preparation* in the *Assay*.

**Test solution**—Quantitatively dilute an accurately measured volume of the *Test stock solution* with *Diluent* to obtain a solution having a concentration of about 100 µg of bisoprolol fumarate per mL.

**Chromatographic system** (see *Chromatography* (621))—Prepare as directed in the *Assay*, but use a 260-nm detector. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between chlorothiazide and hydrochlorothiazide is not less than 1.5. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.3; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$(100/F)(W_B / W_H)(C_S / C_B)(r_1 / r_3)$$

in which *F* is the response factor, equal to 1.2 for the peak with a relative retention time of 0.69 and 1.4 for the peak with a relative retention time of 1.2, both retention times relative to that of the hydrochlorothiazide peak; *W<sub>B</sub>* and *W<sub>H</sub>* are the labeled quantities, in mg, of bisoprolol fumarate and hydrochlorothiazide, respectively, in each Tablet; *C<sub>S</sub>* is the concentration, in mg per mL, of USP Hydrochlorothiazide RS in the *Standard solution*; *C<sub>B</sub>* is the concentration, in mg per mL, of bisoprolol fumarate in the *Test solution*; *r<sub>1</sub>* is the peak response of each of the two impurities obtained from the *Test solution*; and *r<sub>3</sub>* is the response for the hydrochlorothiazide peak obtained from the *Standard solution*: not more than 1.0% for the impurity with a relative retention time of 0.69 is found; and not more than 2.0% for the impurity with a relative retention time of 1.2 is found.

#### Assay—

**Diluent**—Mix 10 mL of 1 M dibutylammonium phosphate with 1000 mL of a mixture of water and acetonitrile (1:1).

**Solution A**—Mix 10 mL of 1 M dibutylammonium phosphate with 1000 mL of water.

**Solution B**—Prepare a mixture of acetonitrile and water (3:2). Add 10 mL of 1 M dibutylammonium phosphate per liter, stir vigorously for 2 minutes, filter, and degas.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Prepare a solution of USP Chlorothiazide RS and USP Hydrochlorothiazide RS in *Diluent* containing 40 µg of each per mL.

**Standard preparation**—Dissolve suitable quantities of USP Bisoprolol Fumarate RS and USP Hydrochlorothiazide RS in *Diluent* to obtain a solution having known concentrations of about 100 µg of each per mL. Stir by mechanical means for 1 hour.

**Assay stock preparation**—Weigh 10 Tablets, and transfer to a 100-mL volumetric flask. Add about 50 mL of *Diluent*, sonicate for 10 minutes, and cool. Dilute with *Diluent* to volume, stir by mechanical means for 1 hour, and centrifuge.

**Bisoprolol fumarate assay preparation**—Quantitatively transfer a portion of the *Assay stock preparation* to a 50-mL volumetric flask, and dilute with *Diluent* to volume to obtain a solution having a concentration of about 100 µg of bisoprolol fumarate per mL.

**Hydrochlorothiazide assay preparation**—Quantitatively transfer a portion of the *Assay stock preparation* to a 50-mL

volumetric flask, and dilute with *Diluent* to volume to obtain a solution having a concentration of about 62.5 µg of hydrochlorothiazide per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with 225-nm detector and an 8-mm × 10-cm column that contains packing L11. The flow rate is about 3 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	100	0	equilibration
0–9.0	100→40	0→60	linear gradient
9.0–9.1	40→100	60→0	linear gradient
9.1–12.0	100	0	re-equilibration

Chromatograph the *System suitability solution*, and record the peak areas as directed for *Procedure*: the resolution, *R*, between chlorothiazide and hydrochlorothiazide is not less than 1.5. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the tailing factor for the hydrochlorothiazide peak is not more than 1.3; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation*, *Bisoprolol fumarate assay preparation*, and *Hydrochlorothiazide assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantities, in mg, of bisoprolol fumarate ( $C_{18}H_{31}NO_4$ )<sub>2</sub> ·  $C_4H_4O_4$  and hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) in the portion of Tablets taken by the formula:

$$5000(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Bisoprolol Fumarate RS or USP Hydrochlorothiazide RS in the *Standard preparation*, as appropriate; *V* is the volume of the *Assay stock preparation* used to prepare the *Bisoprolol fumarate assay preparation* or the *Hydrochlorothiazide assay preparation*; *r<sub>U</sub>* is the peak area obtained from the *Bisoprolol fumarate assay preparation* or the *Hydrochlorothiazide assay preparation*, as appropriate; and *r<sub>S</sub>* is the corresponding peak area obtained from the *Standard preparation*.

## Bleomycin for Injection

» Bleomycin for Injection contains an amount of Bleomycin Sulfate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of bleomycin.

#### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for constitution* (CN 1-May-2017).

#### USP Reference standards (11)—

USP Bleomycin Sulfate RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.



**Identification—**

**A:** *Infrared Absorption* (197K).

**B:** It responds to the tests for *Sulfate* (191).

**Bacterial Endotoxins Test** (85)—It contains not more than 10.0 USP Endotoxin Units per Bleomycin Unit.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, the entire contents of each container being used.

**Water Determination, Method 1c** (921): not more than 6.0%. Prepare the specimen for test as follows. Use a dry syringe to inject 4 mL of anhydrous methanol through the stoppers of two tared containers, respectively, and shake to dissolve. Using the same syringe, aspirate the contents of the two containers, transfer to the titration vessel, and titrate. Perform a blank determination on 8 mL of the anhydrous methanol. Determine the weights of the empty containers, and calculate the percentage of water.

**Other requirements**—It meets the requirements for *pH*, *Copper*, and *Content of bleomycins* under *Bleomycin Sulfate*. It meets also the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

**Change to read:****Assay—**

**Assay preparation**—Constitute Bleomycin for Injection as directed in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Buffer B.16* (CN.1-May-2017) to obtain a solution having a convenient concentration.

**Procedure**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with *Buffer B.16* (CN.1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

**Bleomycin Sulfate**

Bleomycin sulfate (salt).

Bleomycin sulfate (salt) [9041-93-4].

» Bleomycin Sulfate is the sulfate salt of bleomycin, a mixture of basic cytotoxic glycopeptides produced by the growth of *Streptomyces verticillus*, or produced by other means. It has a potency of not less than 1.5 Bleomycin Units and not more than 2.0 Bleomycin Units per mg.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Bleomycin Sulfate RS

USP Endotoxin RS

**Identification—**

**A:** *Infrared Absorption* (197K).

**B:** It responds to the tests for *Sulfate* (191).

**pH** (791): between 4.5 and 6.0, in a solution containing 10 Bleomycin Units per mL.

**Loss on drying** (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 6.0% of its weight.

**Copper—**

**Dilute nitric acid**—Dilute 20 mL of nitric acid to 2000 mL with water.

**Copper stock solution**—Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, dilute with *Dilute nitric acid* to volume, and mix. Store in a polyethylene bottle. This solution contains 1000 µg of copper per mL.

**Standard preparations**—Transfer 5.0 mL of *Copper stock solution* to a 100-mL volumetric flask, dilute with *Dilute nitric acid* to volume, and mix. Transfer 3.0, 9.0, and 15.0 mL, respectively, of this solution to separate 100-mL volumetric flasks, dilute the contents of each flask with *Dilute nitric acid* to volume, and mix. These *Standard preparations* contain, respectively, 1.5, 4.5, and 7.5 µg of copper per mL.

**Test preparation**—Dissolve about 75 mg of Bleomycin Sulfate, accurately weighed, in 10.0 mL of *Dilute nitric acid*.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparations* and the *Test preparation* at the copper emission line at 324.8 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a copper hollow-cathode lamp and an air-acetylene flame, using *Dilute nitric acid* as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of copper, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration, *C*, in µg per mL, of copper in the *Test preparation*. Calculate the percentage of copper in the portion of Bleomycin Sulfate taken by the formula:

$$C / W$$

in which *W* is the weight, in mg, of Bleomycin Sulfate taken to prepare the *Test preparation*: not more than 0.1% is found.

**Content of bleomycins—**

**Mobile phase**—Dissolve 960 mg of sodium 1-pentane-sulfonate in 1000 mL of deaerated 0.08 N acetic acid, adjust with ammonium hydroxide to a pH of 4.3, filter, and degas. [NOTE—1.86 g of edetate disodium may be included if needed to obtain satisfactory chromatography.] Use a linear gradient of 10% to 40% methanol mixed with this solution, with a gradient mixing time of 60 minutes, and allow chromatography to proceed with the final gradient mixture for a further 20 minutes or until demethylbleomycin A<sub>2</sub> has been eluted.

**Test preparation**—Dissolve Bleomycin Sulfate in deaerated water to obtain a solution having a concentration of about 2.5 Bleomycin Units per mL. Store this solution in a refrigerator until just prior to use.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 250-mm stainless steel column containing packing L1. The flow rate is about 1.2 mL per minute.

**Procedure**—Inject about 10 µL of the *Test preparation* into the chromatograph by means of a suitable microsyringe or sampling valve, record the chromatogram, and measure the peak responses for all peaks. The elution order is bleomycinic acid, bleomycin A<sub>2</sub> (major peak), bleomycin A<sub>3</sub>, bleomycin B<sub>2</sub> (major peak), bleomycin B<sub>4</sub>, and demethylbleomycin A<sub>2</sub>. Calculate the percentage contents of bleomycin A<sub>2</sub>, bleomycin B<sub>2</sub>, and bleomycin B<sub>4</sub> taken by the formula:

$$100r_i / r_t$$

in which *r<sub>i</sub>* is the peak response corresponding to the particular bleomycin and *r<sub>t</sub>* is the total of the responses of all peaks: the content of bleomycin A<sub>2</sub> is between 55% and 70%; the content of bleomycin B<sub>2</sub> is between 25% and 32%; the content of bleomycin B<sub>4</sub> is not more than 1%; and the combined percentage of bleomycin A<sub>2</sub> and bleomycin B<sub>2</sub> is not less than 90%.



**Other requirements**—Where the label states that Bleomycin Sulfate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Bleomycin for Injection*. Where the label states that Bleomycin Sulfate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Bleomycin for Injection*.

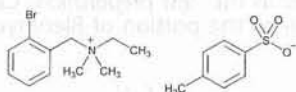
#### Change to read:

#### Assay—

**Assay preparation**—Dissolve a suitable quantity of Bleomycin Sulfate, accurately weighed, in *Buffer B.16* (CN 1-May-2017), and quantitatively dilute with *Buffer B.16* (CN 1-May-2017) to obtain a solution having a convenient concentration.

**Procedure**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with *Buffer B.16* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

### Bretylum Tosylate



$C_{18}H_{24}BrNO_3S$  414.36

Benzenemethanaminium, 2-bromo-N-ethyl-N,N-dimethyl-, salt with 4-methylbenzenesulfonic acid (1:1). (o-Bromobenzyl)ethyldimethylammonium *p*-toluenesulfonate [61-75-6].

» Bretylum Tosylate contains not less than 98.0 percent and not more than 101.0 percent of  $C_{18}H_{24}BrNO_3S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

#### USP Reference standards (11)—

USP Bretylum Tosylate RS

#### Identification—

A: *Infrared Absorption* (197K).

B: The retention time of the major peak in the chromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Related compounds*.

**Loss on drying** (731)—Dry it in vacuum at 75° for 2 hours; it loses not more than 3.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

#### Delete the following:

• **Heavy metals**, *Method I* (231): 0.002%. (Official 1-Jan-2018)

#### Related compounds—

0.01 M Sodium 1-octanesulfonate solution—Dissolve 1.0814 g of 1-sodium octanesulfonate in 500 mL of water.

**Mobile phase**—Prepare a mixture of 0.01 M Sodium 1-octanesulfonate solution, acetonitrile, glacial acetic acid, and triethylamine (81:19:2:0.5). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Dissolve an accurately weighed quantity of USP Bretylum Tosylate RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 20 µg per mL.

**Test solution**—Transfer about 200 mg of Bretylum Tosylate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 265-nm detector and a 4.6-mm × 25-cm column that contains packing L11. The flow rate is about 1.9 mL per minute. Chromatograph the *Standard solution*, record the chromatograms, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 30 µL) of the *Test solution* and the *Standard solution* into the chromatograph, record the chromatograms, and measure the peak responses. The relative retention times are about 0.25, 0.74, 1.0, 1.27, 1.40 for tosylate ion, o-bromobenzyltrimethylamine, bretylum, m-bromobenzyltrimethylamine, and p-bromobenzyltrimethylamine, respectively. The sum of the responses for all the peaks, excluding those of the bretylum and tosylate peaks, from the *Test solution* is not more than two times the bretylum response from the *Standard solution* (2%); and no individual peak response is greater than that of the bretylum peak from the *Standard solution* (1%).

**Assay**—Dissolve about 300 mg of Bretylum Tosylate, accurately weighed, in 50 mL of dioxane in a conical flask. Add 2 drops of crystal violet TS, and titrate with 0.025 N perchloric acid in dioxane to a blue-green endpoint. Perform a blank determination (see *Titrimetry* (541)), and make any necessary correction. Each mL of 0.025 N perchloric acid is equivalent to 10.36 mg of  $C_{18}H_{24}BrNO_3S$ .

### Bretylum Tosylate Injection

» Bretylum Tosylate Injection is a sterile solution of Bretylum Tosylate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{18}H_{24}BrNO_3S$ .

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass.

#### USP Reference standards (11)—

USP Bretylum Tosylate RS

USP Endotoxin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of bretylum tosylate.

**pH** (791): between 3.5 and 7.0.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

#### Assay—

**pH 3.1 Tetramethylammonium phosphate buffer**—Dissolve 1.38 g of monobasic sodium phosphate and 2.0 mL of 25% tetramethylammonium hydroxide solution in methanol in 800 mL of water, adjust with phosphoric acid to a pH of 3.1 ± 0.1, dilute with water to 1000 mL, and mix.

**Mobile phase**—Transfer 15 mL of tetrahydrofuran and 75 mL of acetonitrile to a 1000-mL volumetric flask, and dilute with pH 3.1 Tetramethylammonium phosphate buffer to volume.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bretylum Tosylate RS in water, and dilute



quantitatively, and stepwise if necessary, with water to obtain a solution having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of bretylium tosylate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for tosylate and 1.0 for bretylium; the resolution,  $R$ , between the bretylium and tosylate peaks is not less than 3.0; and the relative standard deviation for replicate injections is not more than 1.4%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{18}H_{24}BrNO_3S$  in each mL of the Injection taken by the formula:

$$50(C/V)(r_u/r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Bretylium Tosylate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $r_u$  and  $r_s$  are the bretylium peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bretylium Tosylate in Dextrose Injection

» Bretylium Tosylate in Dextrose Injection is a sterile solution of Bretylium Tosylate and Dextrose in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amounts of bretylium tosylate ( $C_{18}H_{24}BrNO_3S$ ) and dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ). It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.

**USP Reference standards** (11)—

USP Bretylium Tosylate RS

USP Endotoxin RS

**Identification**—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for bretylium tosylate*.

**B:** It responds to the *Identification* test under *Dextrose*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of bretylium tosylate.

**pH** (791): between 3.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay for bretylium tosylate**—

**pH 3.1 Tetramethylammonium phosphate buffer, Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay under Bretylium Tosylate Injection*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of bretylium

tosylate, to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of bretylium tosylate ( $C_{18}H_{24}BrNO_3S$ ) in each mL of the Injection taken by the formula:

$$50(C/V)(r_u/r_s)$$

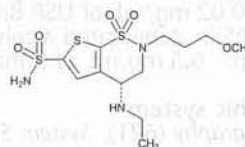
in which  $C$  is the concentration, in mg per mL, of USP Bretylium Tosylate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $r_u$  and  $r_s$  are the bretylium peak responses from the *Assay preparation* and the *Standard preparation*, respectively.

**Assay for dextrose**—Transfer an accurately measured volume of Injection, containing 2 to 5 g of dextrose, to a 100-mL volumetric flask. Add 0.2 mL of 6 N ammonium hydroxide, dilute with water to volume, and mix. Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* (781)). Calculate the percentage (g per 100 mL) of dextrose ( $C_6H_{12}O_6 \cdot H_2O$ ) in the portion of Injection taken by the formula:

$$(100/52.9)(198.17/180.16)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees; 198.17 and 180.16 are the molecular weights for dextrose monohydrate and anhydrous dextrose, respectively;  $A$  is 100 mm divided by the length of the polarimeter tube, in mm; and  $R$  is the observed rotation, in degrees.

## Brinzolamide



$C_{12}H_{21}N_3O_5S_3$  383.51  
2H-Thieno[3,2-e]-1,2-thiazine-6-sulfonamide, 4-(ethylamino)-3,4-dihydro-2-(3-methoxypropyl)-, 1,1-dioxide, (R)-; (R)-4-(Ethylamino)-3,4-dihydro-2-(3-methoxypropyl)-2H-thieno[3,2-e]-1,2-thiazine-6-sulfonamide 1,1-dioxide [138890-62-7].

### DEFINITION

Brinzolamide contains NLT 98.0% and NMT 102.0% of brinzolamide ( $C_{12}H_{21}N_3O_5S_3$ ), calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *System suitability solution*, as obtained in *Limit of Brinzolamide Related Compound A*.

### ASSAY

#### PROCEDURE

**Buffer:** Add 4.0 mL of triethylamine to 1000 mL of water, and adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Acetonitrile and Buffer (25:75)

**Standard solution:** 0.1 mg/mL of USP Brinzolamide RS in *Mobile phase*

**Sample solution:** 0.1 mg/mL of Brinzolamide in *Mobile phase*



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 μL

**System suitability**Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1200 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of brinzolamide

(C<sub>12</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>S<sub>3</sub>) in the portion of Brinzolamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Brinzolamide RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Brinzolamide in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**Delete the following:**

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-

(Jan-2018)

- **LIMIT OF BRINZOLAMIDE RELATED COMPOUND A**

Mobile phase: Dehydrated alcohol, chromatographic hexane, methanol, and diethylamine (55: 40: 5: 0.2)

System suitability solution: 0.4 mg/mL of USP Brinzolamide RS and 0.02 mg/mL of USP Brinzolamide Related Compound A RS in dehydrated alcohol

Sample solution: 0.5 mg/mL of Brinzolamide in dehydrated alcohol

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L51

Flow rate: 0.75 mL/min

Injection volume: 5 μL

**System suitability**Sample: *System suitability solution*

[NOTE—The relative retention times for brinzolamide and brinzolamide related compound A are 1.0 and 1.2, respectively.]

Suitability requirements

Resolution: NLT 1.8 between brinzolamide and brinzolamide related compound A peaks

Column efficiency: NLT 2000 theoretical plates for the brinzolamide peak

Tailing factor: NMT 1.8 for the brinzolamide peak

**Analysis**Sample: *Sample solution*

Calculate the percentage of brinzolamide related compound A in the portion of Brinzolamide taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak response for brinzolamide related compound A $r_T$  = sum of the peak responses for brinzolamide and brinzolamide related compound A

Acceptance criteria: NMT 0.5%

• **ORGANIC IMPURITIES**Buffer: Prepare as directed in the *Assay*.Mobile phase A: Prepare as directed for *Mobile phase* in the *Assay*.

Mobile phase B: Acetonitrile and Buffer (35:65)

System suitability solution: 0.1 mg/mL each of USP Brinzolamide RS and USP Brinzolamide Related Compound B RS in *Mobile phase A*Sample solution: 1 mg/mL of Brinzolamide in *Mobile phase A***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.0 mL/min

Injection volume: 10 μL

**System suitability**Sample: *System suitability solution*Use *Mobile phase A*.

[NOTE—The relative retention times for brinzolamide related compound B and brinzolamide are 0.8 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.0 between the brinzolamide and brinzolamide related compound B peaks

Column efficiency: NLT 1200 theoretical plates for the brinzolamide peak

Tailing factor: NMT 2.0 for the brinzolamide peak

**Analysis 1**Use *Mobile phase A*.Sample: *Sample solution*Allow the elution to continue for 20 min, and measure the areas for all the peaks, excluding the peaks of *Mobile phase A*.

Calculate the percentage of each impurity in the portion of Brinzolamide taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak response for each impurity $r_T$  = sum of all the peak responses

Acceptance criteria 1: NMT 0.3% for any individual impurity

**Analysis 2**Use *Mobile phase B*.Sample: *Sample solution*

Allow the elution to continue for 20 min, and measure the areas for brinzolamide and all the peaks having a relative retention greater than 6.

Calculate the percentage of each impurity in the portion of Brinzolamide taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak response for each impurity $r_T$  = sum of all the peak responsesAcceptance criteria 2: NMT 0.3% for any individual impurity; NMT 1.0% for total impurities from *Analysis 1* and *Analysis 2***SPECIFIC TESTS**

- **LOSS ON DRYING** (731)

Analysis: Dry under vacuum at 100°–105° for 3 h.

Acceptance criteria: NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS** (11)

USP Brinzolamide RS

USP Brinzolamide Related Compound A RS

Brinzolamide (S)-isomer.

C<sub>12</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>S<sub>3</sub> 383.52



USP Brinzolamide Related Compound B RS  
(*R*-4-Amino)-2,3-dihydro-2-(3-methoxypropyl)-4*H*-thieno  
[3,2,-*e*]-thiazine-6-sulfonamide-1,1-dioxide ethandioate  
1:1.  
 $C_{10}H_{17}N_3O_5S_3 \cdot C_2H_2O_4$  445.49

## Brinzolamide Ophthalmic Suspension

### DEFINITION

Brinzolamide Ophthalmic Suspension is a sterile, aqueous suspension of Brinzolamide containing a suitable antimicrobial preservative. It contains NLT 90.0% and NMT 110.0% of the labeled amount of brinzolamide ( $C_{12}H_{21}N_3O_5S_3$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of *Standard solution A*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 11.75 g/L of ammonium acetate in water. Adjust with acetic acid to a pH of 5.2.

**Mobile phase:** Methanol and *Buffer* (35:65)

**Standard solution A:** 0.2 mg/mL of USP Brinzolamide RS in *Mobile phase*

**System suitability solution:** 0.06 mg/mL of USP Brinzolamide Related Compound B RS in *Standard solution A*

**Sample solution:** Nominally 0.2 mg/mL of brinzolamide in *Mobile phase* prepared as follows. Transfer a volume of Ophthalmic Suspension, equivalent to 10 mg of brinzolamide, into a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 μL

#### System suitability

**Samples:** *Standard solution A* and *System suitability solution*

[NOTE—The relative retention times for brinzolamide related compound B are between 0.48 and 0.61, and the relative retention time for brinzolamide is 1.0.]

#### Suitability requirements

**Resolution:** NLT 4.5 between the brinzolamide and brinzolamide related compound B peaks, *System suitability solution*

**Tailing factor:** NMT 2.0, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution A*

#### Analysis

**Samples:** *Standard solution A* and *Sample solution*

Calculate the percentage of the labeled amount of brinzolamide ( $C_{12}H_{21}N_3O_5S_3$ ) in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from *Standard solution A*

$C_S$  = concentration of USP Brinzolamide RS in *Standard solution A* (mg/mL)

$C_U$  = nominal concentration of brinzolamide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### IMPURITIES

#### LIMIT OF BRINZOLAMIDE RELATED COMPOUND A

**Mobile phase:** Dehydrated alcohol, chromatographic hexane, methanol, and diethylamine (55: 40: 5: 0.2)

**System suitability solution:** 0.4 mg/mL of USP Brinzolamide RS and 0.02 mg/mL of USP Brinzolamide Related Compound A RS in dehydrated alcohol

**Sample solution:** Transfer a volume of Ophthalmic Suspension, equivalent to 10 mg of brinzolamide, to a 25-mL volumetric flask. Dilute with alcohol to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L51

**Flow rate:** 0.75 mL/min

**Injection volume:** 5 μL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for brinzolamide and brinzolamide related compound A are 1.0 and 1.2, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.8 between the brinzolamide and brinzolamide related compound A peaks

**Column efficiency:** NLT 2000 theoretical plates for the brinzolamide peak

**Tailing factor:** NMT 1.8 for the brinzolamide peak

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of brinzolamide related compound A in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for brinzolamide related compound A

$r_T$  = sum of the peak responses for brinzolamide and brinzolamide related compound A

**Acceptance criteria:** NMT 1.5%

#### ORGANIC IMPURITIES

**Buffer, Mobile phase, Standard solution A, System suitability solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution B:** 2.5 μg/mL of USP Brinzolamide Related Compound B RS in *Mobile phase*

#### Analysis

**Samples:** *Sample solution* and *Standard solution B*

Calculate the percentage of each impurity in the portion of Ophthalmic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response for brinzolamide related compound B from *Standard solution B*

$C_S$  = concentration of USP Brinzolamide Related Compound B RS in *Standard solution B* (mg/mL)

$C_U$  = nominal concentration of brinzolamide in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of des-ethyl brinzolamide, 356.46

$M_{r2}$  = molecular weight of des-ethyl brinzolamide oxalate, 445.49



## Acceptance criteria

Any individual impurity: NMT 0.5%

Total impurities: NMT 2.0%

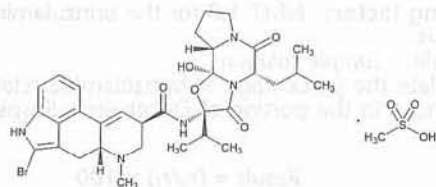
## SPECIFIC TESTS

- **STERILITY TESTS** (71): It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **pH** (791): 6.5–8.5

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at a temperature between 4° and 30°.
- **USP REFERENCE STANDARDS** (11)
  - USP Brinzolamide RS
  - USP Brinzolamide Related Compound A RS  
Brinzolamide (S)-isomer.  
 $C_{12}H_{21}N_3O_5S_3$  383.52
  - USP Brinzolamide Related Compound B RS  
(R-4-Amino)-2,3-dihydro-2-(3-methoxypropyl)-4H-thieno[3,2,-e]-thiazine-6-sulfonamide-1,1-dioxide ethandioate 1:1.  
 $C_{10}H_{17}N_3O_5S_3 \cdot C_2H_2O_4$  445.49

## Bromocriptine Mesylate



$C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$  750.70  
 Ergotaman-3',6',18-trione, 2-bromo-12'-hydroxy-2'-(1-methylethyl)-5'-(2-methylpropyl)-, monomethanesulfonate (salt), (5 $\alpha$ )-;  
 2-Bromoergocryptine monomethanesulfonate (salt) [22260-51-1].

## DEFINITION

Bromocriptine Mesylate contains NLT 98.0% and NMT 102.0% of  $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ , calculated on the dried basis.

## IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M): Undried
- **B. ULTRAVIOLET ABSORPTION** (197U)  
 Sample solution: 50  $\mu$ g/mL in 0.1 M methanolic methanesulfonic acid  
 Acceptance criteria: Meets the requirements

## ASSAY

## PROCEDURE

**Sample solution:** 600 mg of Bromocriptine Mesylate  
**Analysis:** Dissolve with 80 mL of a mixture of acetic anhydride and glacial acetic acid (7:1). Titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 75.07 mg of  $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ .

Acceptance criteria: 98.0%–102.0% on the dried basis

## IMPURITIES

## Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.1%

## Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-Jan-2018)

## Organic Impurities

## PROCEDURE 1: LIMIT OF METHANESULFONIC ACID CONTENT

**Sample solution:** 400 mg of Bromocriptine Mesylate  
**Analysis:** Dissolve with 70 mL of methanol. Titrate under nitrogen with 0.1 N methanolic potassium hydroxide VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N methanolic potassium hydroxide is equivalent to 9.61 mg of  $CH_3SO_3H$ .  
**Acceptance criteria:** NLT 12.5% and NMT 13.4% of  $CH_3SO_3H$  on the dried basis

## PROCEDURE 2

**Solution A:** 0.1 N citric acid solution. Adjust with hydrochloric acid to a pH of 2.0.

**Diluent:** Methanol and *Solution A* (1:1)

**Solution B:** Acetonitrile and 0.01 M phosphate buffer, pH 7.0 (2:3)

**Solution C:** Acetonitrile and 0.01 M phosphate buffer, pH 7.0 (3:2)

**Mobile phase:** See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
18	100	0
30	0	100
40	0	100
41	100	0

**System suitability solution:** 2.0 mg/mL each of  $\alpha$ -ergocryptine and Bromocriptine Mesylate in *Diluent*

**Standard stock solution:** 46  $\mu$ g/mL of USP Bromocriptine Mesylate RS in methanol and *Solution A* (1:1).

[NOTE—Dissolve in 50% of the flask volume of methanol and dilute with *Solution A* to volume.]

**Standard solution:** 4.6  $\mu$ g/mL of USP Bromocriptine Mesylate RS in *Diluent* from the *Standard stock solution*

**Sample solution:** 4.6 mg/mL of Bromocriptine Mesylate in methanol and *Solution A* (1:1). [NOTE—Dissolve in 50% of the flask volume of methanol and dilute with *Solution A* to volume.]

## Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Column:** 4.6-mm  $\times$  15-cm; 3- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20  $\mu$ L

## System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for  $\alpha$ -ergocryptine and bromocriptine mesylate are 0.46 and 1.0, respectively.]

## Suitability requirements

**Resolution:** NLT 15 between  $\alpha$ -ergocryptine and bromocriptine mesylate, *System suitability solution*

**Tailing factor:** NMT 1.5, *System suitability solution*

**Relative standard deviation:** NMT 10.0%, *Standard solution*



**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for bromocriptine and bromocriptinine are 1.0 and 1.7, respectively.]

Calculate the percentage of each impurity in the portion of Bromocriptine Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

- $r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of bromocriptine from the *Standard solution*  
 $C_S$  = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Bromocriptine Mesylate in the *Sample solution* (mg/mL)  
 $F$  = relative response factor equal to 1.4 for any peak eluting at a relative retention time of about 0.9 or less, and equal to 1.0 for all other peaks

**Acceptance criteria**

**Individual impurities:** NMT 0.4% of bromocriptinine is found; NMT 0.1% of any individual impurity is found.

**Total impurities:** NMT 1.0%

**SPECIFIC TESTS**• **COLOR OF SOLUTION (631)**

**Matching solutions:** Prepare three solutions, A, B, and C, containing, respectively, the following parts of cobaltous chloride CS, ferric chloride CS, cupric sulfate CS, and dilute hydrochloric acid (1 in 40).

A: 3.0: 3.0: 2.4: 31.6

B: 1.0: 2.4: 0.4: 36.2

C: 0.6: 2.4: 0: 37.0

**Sample solution:** 10 mg/mL of Bromocriptine Mesylate in methanol

**Analysis:** Compare the *Sample solution* with 10-mL portions of the *Matching solutions* in suitable matched tubes.

**Acceptance criteria:** The solution is clear and not darker in color than *Matching solutions* A, B, and C.

• **OPTICAL ROTATION, Specific Rotation (781S)**

**Sample solution:** 10 mg/mL, in a mixture of methylene chloride and methanol (1:1)

**Acceptance criteria:** +95° to +105°

• **LOSS ON DRYING**

(See *Thermal Analysis* (891).)

**Analysis:** Determine the percentage of volatile substances by thermogravimetric analysis using 10 mg of Bromocriptine Mesylate. Heat the specimen under test at the rate of 10°/min in an atmosphere of nitrogen at a flow rate of 45 mL/min. Record the thermogram from ambient temperature to 160°.

**Acceptance criteria:** It loses NMT 4.0% of its weight.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, in a cold place.

• **USP REFERENCE STANDARDS (11)**  
USP Bromocriptine Mesylate RS

**Bromocriptine Mesylate Capsules****DEFINITION**

Bromocriptine Mesylate Capsules contain bromocriptine mesylate ( $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ ) equivalent to NLT 90.0%

and NMT 110.0% of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ).

**IDENTIFICATION**

- **A.** The principal spot of the *Sample solution* corresponds, in  $R_f$  value and color, to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

**ASSAY**• **PROCEDURE**

Conduct this procedure without exposure to daylight and with minimum exposure to artificial light.

**Buffer:** 0.125 g/L of ammonium carbonate in water

**Mobile phase:** Acetonitrile and *Buffer* (3:2)

**Standard solution:** 1.0 mg/mL of bromocriptine from USP Bromocriptine Mesylate RS in dehydrated alcohol. Sonicate as needed.

**Sample solution:** 1.0 mg/mL of bromocriptine in methanol, prepared as follows. Remove, as completely as possible, the contents of NLT 10 Capsules. Weigh and determine the average weight per Capsule. Mix the combined contents, and transfer a weighed quantity of the powder, nominally equivalent to 50 mg of bromocriptine, to a 50-mL volumetric flask. Add 30 mL of dehydrated alcohol, and shake for 15 min. Dilute with dehydrated alcohol to volume, mix, and filter. Use this solution without delay.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 300 nm

**Column:** 4-mm × 25-cm; packing L7

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 1000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of bromocriptine, from USP Bromocriptine Mesylate RS, in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of bromocriptine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION (711)**

**Medium:** 0.1 N hydrochloric acid; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 60 min

**Standard solution:** USP Bromocriptine Mesylate RS in *Medium*, at a concentration similar to the *Sample solution*. [NOTE—A volume of alcohol not to exceed 5% of the total volume of the *Standard solution* may be used to bring the Standard into solution before dilution with *Medium*.]

**Sample solution:** Sample per *Dissolution* (711), passed through a glass-fiber filter.



**Instrumental conditions**(See *Fluorescence Spectroscopy* (853).)**Mode:** Fluorometry**Excitation wavelength:** 315 nm**Emission wavelength:** 445 nm**Blank:** Medium**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of bromocriptine mesylate ( $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ ) dissolved.**Tolerances:** NLT 75% (Q) of the labeled amount of bromocriptine mesylate ( $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ ) is dissolved.• **UNIFORMITY OF DOSAGE UNITS** (905)**Procedure for content uniformity**

Protect all solutions from light.

**Diluent:** Dissolve 1.0 g of tartaric acid in 500 mL of water, add 500 mL of methanol, and mix.**Standard solution:** 0.04 mg/mL of USP Bromocriptine Mesylate RS in *Diluent***Sample solution:** Transfer the contents of 1 Capsule into a 25-mL volumetric flask. Add 15 mL of *Diluent*, and shake by mechanical means for 20 min. Dilute with *Diluent* to volume, and mix. Filter, and dilute 10.0 mL of the clear filtrate with *Diluent* to 50.0 mL.**Instrumental conditions**(See *Ultraviolet-Visible Spectroscopy* (857).)**Mode:** UV**Analytical wavelength:** Maximum absorbance (about 306 nm)**Cell:** 1 cm**Blank:** *Diluent***Analysis****Samples:** *Standard solution*, *Sample solution*, and *Blank*Calculate the percentage of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $A_U$  = absorbance of the *Sample solution* $A_S$  = absorbance of the *Standard solution* $C_S$  = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of bromocriptine in the *Sample solution* (mg/mL) $M_{r1}$  = molecular weight of bromocriptine, 654.59 $M_{r2}$  = molecular weight of bromocriptine mesylate, 750.70**Acceptance criteria:** Meet the requirements**IMPURITIES**• **ORGANIC IMPURITIES**Conduct this test without exposure to daylight and with minimum exposure to artificial light. Perform the test rapidly, preparing and spotting the *Sample solution* last.**Standard stock solution:** 2.3 mg/mL of USP

Bromocriptine Mesylate RS in methanol, equivalent to 2 mg/mL of bromocriptine

**Standard solution 1:** 0.06 mg/mL (3.0%) of bromocriptine in methanol, from *Standard stock solution***Standard solution 2:** 0.04 mg/mL (2.0%) of bromocriptine in methanol, from *Standard stock solution***Standard solution 3:** 0.02 mg/mL (1.0%) of bromocriptine in methanol, from *Standard stock solution***Standard solution 4:** 0.01 mg/mL (0.50%) of bromocriptine in methanol, from *Standard stock solution***Sample solution:** 2.0 mg/mL of bromocriptine in methanol, prepared as follows. Transfer a quantity of the

Capsule contents, equivalent to 20 mg of bromocriptine, to a conical flask. Add 10 mL of methanol, and stir by mechanical means for 20 min. Centrifuge the suspension for 10 min at about 3500 rpm. Use the clear supernatant.

**Chromatographic system**(See *Chromatography* (621), *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture**Application volume:** 50  $\mu$ L as 1.5-cm bands**Developing solvent:** Methylene chloride, dioxane, alcohol, and ammonium hydroxide (180:15:5:1)**Spray reagent:** 0.2% o-phthalaldehyde in sulfuric acid**Analysis****Samples:** *Standard stock solution*, *Standard solutions*, and *Sample solution*Develop under the exclusion of light in a tank lined with filter paper, previously equilibrated for 30 min, using *Developing solvent* until the solvent front has moved a distance of 15 cm on the plate. Dry the plate briefly in a current of cold air. Spray evenly with the *Spray reagent*, and view the plate under long-wavelength UV light.**Acceptance criteria:** Any major secondary spot, other than the principal spot, obtained from the *Sample solution* is not greater in size and intensity than the spot obtained from *Standard solution 1* (3.0%). Any remaining spots are not greater in size and intensity than the spot obtained from *Standard solution 3* (1.0%). The sum of the organic impurities is NMT 5.0%.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.• **USP REFERENCE STANDARDS** (11)  
USP Bromocriptine Mesylate RS**Bromocriptine Mesylate Tablets****DEFINITION**Bromocriptine Mesylate Tablets contain bromocriptine mesylate ( $C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$ ) equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ).**IDENTIFICATION**• **A.** The principal spot of the *Sample solution* corresponds, in  $R_f$  value and color, to that of the *Standard stock solution*, as obtained in the test for *Organic Impurities*.**ASSAY**• **PROCEDURE****Buffer:** 0.01 M ammonium carbonate in water**Mobile phase:** Acetonitrile and *Buffer* (65:35)**Standard solution:** 0.22 mg/mL of USP Bromocriptine Mesylate RS in methanol**Sample solution:** Transfer a quantity of powdered Tablets (NLT 20), equivalent to 10 mg of bromocriptine, to an appropriate container. Add 40 mL of methanol, and stir for 20 min, protected from light. Quantitatively filter through a fine glass filtering funnel into a 50-mL volumetric flask. Rinse the filter with methanol, adding the rinsing to the filtrate, and dilute with methanol to volume.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 300 nm

Column: 4-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 50 µL

**System suitability**Sample: *Standard solution***Suitability requirements**

Coefficient of variation: NMT 3.0% for 3 replicate injections

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of bromocriptine in the *Sample solution* (mg/mL) $M_{r1}$  = molecular weight of bromocriptine, 654.59 $M_{r2}$  = molecular weight of bromocriptine mesylate, 750.70

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION (711)****Test 1:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.**Medium:** 0.1 N hydrochloric acid; 500 mL**Apparatus 1:** 120 rpm**Time:** 60 min**Standard solution:** USP Bromocriptine Mesylate RS at a known concentration in *Medium*[NOTE—A volume of alcohol not to exceed 5% of the total volume of the *Standard solution* may be used to dissolve the Standard before dilution with *Medium*.]**Sample solution:** Sample per *Dissolution* (711), passed through a glass-fiber filter.**Blank:** *Medium***Instrumental conditions**(See *Fluorescence Spectroscopy* (853).)**Mode:** Fluorometry**Excitation wavelength:** 315 nm**Emission wavelength:** 445 nm**Analysis**Samples: *Standard solution*, *Sample solution*, and *Blank*Calculate the percentage of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) dissolved.**Tolerances:** NLT 80% (Q) of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) is dissolved.**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.**Medium:** 0.1 N hydrochloric acid; 500 mL**Apparatus 2:** 50 rpm**Time:** 30 min**Buffer:** 0.01 M ammonium carbonate in water**Mobile phase:** Acetonitrile and *Buffer* (65:35)**Standard solution:** Dissolve USP Bromocriptine Mesylate RS in methanol, and quantitatively dilute with *Medium* to obtain a solution having a known concentration similar to the expected concentration of the *Sample solution*.**Sample solution:** Sample per *Dissolution* (711).**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 300 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 100 µL

**System suitability**Sample: *Standard solution***Suitability requirements**

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) dissolved.**Tolerances:** NLT 80% (Q) of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) is dissolved.**• UNIFORMITY OF DOSAGE UNITS (905)****Procedure for content uniformity**

[NOTE—Protect all solutions from light.]

**Diluent:** Dissolve 1.0 g of tartaric acid in 500 mL of water, add 500 mL of methanol, and mix.**Standard solution:** 0.04 mg/mL of USP Bromocriptine Mesylate RS in *Diluent***Sample solution:** Transfer 1 Tablet into a 25-mL volumetric flask. Add 15 mL of *Diluent*, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume, and mix. Filter, and dilute 10.0 mL of the clear filtrate with *Diluent* to 50.0 mL.**Instrumental conditions**(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

**Analytical wavelength:** 306 nm

Cell: 1 cm

**Blank:** *Diluent***Analysis**Samples: *Standard solution*, *Sample solution*, and *Blank*Calculate the percentage of the labeled amount of bromocriptine ( $C_{32}H_{40}BrN_5O_5$ ) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $A_U$  = absorbance of the *Sample solution* $A_S$  = absorbance of the *Standard solution* $C_S$  = concentration of USP Bromocriptine Mesylate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of bromocriptine in the *Sample solution* (mg/mL) $M_{r1}$  = molecular weight of bromocriptine, 654.59 $M_{r2}$  = molecular weight of bromocriptine mesylate, 750.70

Acceptance criteria: Meet the requirements

**IMPURITIES****• ORGANIC IMPURITIES**[NOTE—Conduct this test without exposure to daylight and with minimum exposure to artificial light. Perform the test rapidly, preparing and spotting the *Sample solution* last.]**Standard stock solution:** 1.2 mg/mL of USP Bromocriptine Mesylate RS in methanol, equivalent to 1 mg/mL of bromocriptine**Standard solution 1:** 0.50 mg/mL (5%) of bromocriptine in methanol, from *Standard stock solution***Standard solution 2:** 0.30 mg/mL (3%) of bromocriptine in methanol, from *Standard stock solution***Standard solution 3:** 0.10 mg/mL (1%) of bromocriptine in methanol, from *Standard stock solution***Sample solution:** Transfer an equivalent to 20 mg of bromocriptine, from powdered Tablets, to a conical flask. Add 10 mL of methanol, and mix for 20 min. Centrifuge the suspension for 10 min at 4000 rpm. Use the clear supernatant.



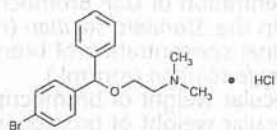
**Chromatographic system**(See *Chromatography* (621), *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture**Application volumes****Standard solutions:** 10-μL, as 1.5-cm bands**Sample solution:** 50-μL, as 1.5-cm bands**Developing solvent system:** Methylene chloride, dioxane, alcohol, and ammonium hydroxide (180: 15: 5: 0.1)**Spray reagent:** 0.2% o-phthalaldehyde in sulfuric acid Analysis**Samples:** *Standard stock solution, Standard solutions, and Sample solution*

Proceed as directed in *Chromatography* (621), *Thin-Layer Chromatography*. Dry the plate for 5 min in a current of cold air. Develop in a tank lined with filter paper, previously equilibrated for 20 min, using *Developing solvent system* until the solvent front has moved a distance of 10 cm on the plate. Dry the plate under vacuum at room temperature for 15 min. Spray evenly with the *Spray reagent*, and view the plate under long-wavelength UV light.

**Acceptance criteria:** Any spot, other than the principal spot, from the *Sample solution* is not greater in size and intensity than the spot from *Standard solution 2* (3.0%). Any remaining spots are not greater in size and intensity than the spot obtained from *Standard solution 3* (1.0%). The sum of the organic impurities is NMT 5.0%.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** The labeling indicates the *Dissolution* test with which the product complies.
- **USP REFERENCE STANDARDS (11)**  
USP Bromocriptine Mesylate RS

**Bromodiphenhydramine Hydrochloride** $C_{17}H_{20}BrNO \cdot HCl$  370.71Ethanamine, 2-(4-bromophenyl)phenylmethoxy-*N,N*-dimethyl-, hydrochloride.2-(*p*-Bromo- $\alpha$ -phenylbenzyl)oxy-*N,N*-dimethylethylamine hydrochloride [1808-12-4].

» Bromodiphenhydramine Hydrochloride contains not less than 98.0 percent and not more than 101.0 percent of  $C_{17}H_{20}BrNO \cdot HCl$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.**USP Reference standards (11)**—

USP Bromodiphenhydramine Hydrochloride RS

**Identification**—**A:** *Infrared Absorption* (197K).**B:** *Ultraviolet Absorption* (197U)—**Solution:** 15 μg per mL.**Medium:** 0.1 N sulfuric acid.

Absorptivities at 228 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 148° and 152°.**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Assay**—Dissolve about 700 mg of Bromodiphenhydramine Hydrochloride, accurately weighed, in 50 mL of glacial acetic acid, and add 10 mL of benzene and 15 mL of mercuric acetate TS. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 37.07 mg of  $C_{17}H_{20}BrNO \cdot HCl$ .

**Bromodiphenhydramine Hydrochloride Oral Solution**

» Bromodiphenhydramine Hydrochloride Oral Solution contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of bromodiphenhydramine hydrochloride ( $C_{17}H_{20}BrNO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.**USP Reference standards (11)**—

USP Bromodiphenhydramine Hydrochloride RS

**Identification, Infrared Absorption** (197K)—

**Test specimen**—Transfer the final solution obtained from the titration in the *Assay* to a separator, add about 1 mL of 0.1 N sulfuric acid, and shake with 25 mL of ether. (Methyl red enters the ether phase.) Drain the aqueous layer into another separator, add 5 mL of 1 N sodium hydroxide, and shake with 10 mL of chloroform. Drain the chloroform layer into a small flask containing 2 g of anhydrous sodium sulfate, and swirl. Pour the chloroform solution through a small cotton pledget, pre-rinsed with chloroform, into a beaker, and evaporate to about 5 mL. Apply a few drops of the solution directly to a potassium bromide plate, and completely remove the chloroform by warming for 2 to 3 minutes under an IR lamp.

**Alcohol Determination, Method I** (611): between 12.0% and 15.0% of  $C_2H_5OH$ .

**Assay**—Evaporate an accurately measured volume of Oral Solution, equivalent to about 250 mg of bromodiphenhydramine hydrochloride, to about half the original volume, using a suitable vacuum evaporator. Transfer the concentrated solution to a 250-mL separator, with the aid of sufficient warm water to bring the volume to the original volume. Add 20 g of sodium chloride, and shake until dissolved. Add 5 mL of 1 N sodium hydroxide, shake with 100 mL of ether, and drain the aqueous layer into a second separator containing 50 mL of ether. Shake, and discard the aqueous layer. Wash the ether solutions with two 20-mL portions of water, shaking each aqueous portion successively in the two separators, and then discard the aqueous solutions. Extract the ether solutions successively with 10.0 mL of 0.1 N sulfuric acid VS, followed by two 5-mL portions of water, and collect the aqueous extracts in a conical flask. Add methyl red TS to the solution in the flask, and titrate the excess acid with 0.02 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetric*).



try (541)). Each mL of 0.1 N sulfuric acid is equivalent to 37.07 mg of bromodiphenhydramine hydrochloride ( $C_{17}H_{20}BrNO \cdot HCl$ ).

### Bromodiphenhydramine Hydrochloride and Codeine Phosphate Oral Solution

» Bromodiphenhydramine Hydrochloride and Codeine Phosphate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of bromodiphenhydramine hydrochloride ( $C_{17}H_{20}BrNO \cdot HCl$ ) and codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate the alcohol content.

**USP Reference standards** (11)—

USP Bromodiphenhydramine Hydrochloride RS

USP Codeine Phosphate RS

**Identification**—

**A: Thin-Layer Chromatographic Identification Test** (201)—

**Test solution**—Transfer a volume of Oral Solution, equivalent to about 10 mg of codeine phosphate, to a separator, and add 5 mL of water, 5 mL of methylene chloride, and 1 mL of ammonium hydroxide. Shake for 1 minute, allow the layers to separate, and use the clear, lower layer.

**Standard solution**—Prepare a solution of USP Bromodiphenhydramine Hydrochloride RS and USP Codeine Phosphate RS in methanol containing 10 mg of each per mL.

**Developing solvent system**: a mixture of alcohol and ammonium hydroxide (49:1).

**B:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The total aerobic microbial count does not exceed 100 cfu per mL, and the total combined molds and yeasts count does not exceed 50 cfu per mL.

**pH** (791): between 4.5 and 6.5.

**Alcohol Determination, Method II** (611): between 4.0% and 6.0% is found.

**Assay**—

**Diluent**—Prepare a mixture of methanol and water (80:20).

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, water, 0.1 N ammonium hydroxide solution, and 0.1 N ammonium nitrate solution (27:3:2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve accurately weighed quantities of USP Bromodiphenhydramine Hydrochloride RS and USP Codeine Phosphate RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having known concentrations of about 100 µg per mL and 80 µg per mL, respectively.

**Assay preparation**—Using a pipet calibrated “to contain”, transfer an accurately measured volume of Oral Solution, equivalent to about 10 mg of bromodiphenhydramine hy-

drochloride and 8 mg of codeine phosphate, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30.0-cm column that contains packing L3. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for bromodiphenhydramine and 1.4 for codeine; the resolution, *R*, between bromodiphenhydramine and codeine is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for bromodiphenhydramine and codeine. Calculate the quantity, in mg, of bromodiphenhydramine hydrochloride ( $C_{17}H_{20}BrNO \cdot HCl$ ) in each mL of the Oral Solution taken by the formula:

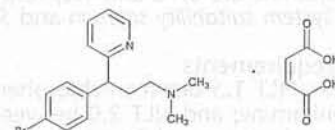
$$100(C/V)(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Bromodiphenhydramine Hydrochloride RS in the *Standard preparation*; *V* is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the bromodiphenhydramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of codeine phosphate hemihydrate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in each mL of the Oral Solution taken by the formula:

$$(406.37/397.36)(100C/V)(r_U / r_S)$$

in which 406.37 and 397.36 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively; *C* is the concentration, in mg per mL, of USP Codeine Phosphate RS in the *Standard preparation*; *V* is the volume, in mL, of Oral Solution taken to prepare the *Assay preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the codeine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Brompheniramine Maleate



$C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$  435.31  
2-Pyridinepropanamine, γ-(4-bromophenyl)-*N,N*-dimethyl-, (±)-, (Z)-2-butenedioate (1:1);  
(±)-2-*p*-Bromo-α-2-(dimethylamino)ethylbenzylpyridine maleate (1:1) [980-71-2].

#### DEFINITION

Brompheniramine Maleate, dried at 105° for 3 h, contains NLT 98.0% and NMT 102.0% of brompheniramine maleate ( $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ ).

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention times of the maleic acid and brompheniramine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.



**ASSAY**• **PROCEDURE**

**Solution A:** 5.44 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0 ± 0.1.

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	95	5
1	95	5
20	70	30
30	70	30
31	95	5
40	95	5

**Diluent:** Acetonitrile and Solution A (5:95)

**System suitability stock solution:** 0.02 mg/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Maleate RS, and USP Chlorpheniramine Related Compound B RS in *Diluent*. Sonicate for 1 min.

**System suitability solution:** 0.5 mg/mL of USP Brompheniramine Maleate RS and 2 µg/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Maleate RS, and USP Chlorpheniramine Related Compound B RS in *Diluent*, prepared as follows. Transfer 5.0 mg of USP Brompheniramine Maleate RS to a 10-mL volumetric flask, add 5.0 mL of *Diluent*, and 1.0 mL of the *System suitability stock solution*, and dilute with *Diluent* to volume.

**Standard solution:** 0.5 mg/mL of USP Brompheniramine Maleate RS in *Diluent*. Sonicate for 1 min.

**Sample solution:** 0.5 mg/mL of Brompheniramine Maleate in *Diluent*. Sonicate for 1 min.

**Chromatographic system**

(See Chromatography <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 225 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

**System suitability**

[NOTE—The relative retention times for maleic acid and brompheniramine are 0.18 and 1.0, respectively.]

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between chlorpheniramine and brompheniramine; and NLT 2.0 between chlorpheniramine related compound B and pheniramine, *System suitability solution*

**Tailing factor:** NMT 2.0 for brompheniramine, *Standard solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of brompheniramine maleate (C<sub>16</sub>H<sub>19</sub>BrN<sub>2</sub> · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) in the portion of Brompheniramine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of brompheniramine from the *Sample solution*

$r_S$  = peak response of brompheniramine from the *Standard solution*

$C_S$  = concentration of USP Brompheniramine Maleate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Brompheniramine Maleate in the *Sample solution* (mg/mL)

**Acceptance criteria:** NLT 98.0%–NMT 102.0% on the previously dried basis

**IMPURITIES**

• **RESIDUE ON IGNITION** (281): NMT 0.2%

• **ORGANIC IMPURITIES**

**Solution A, Solution B, Diluent, Mobile phase, System suitability solution, and Chromatographic system:**

Proceed as directed in the *Assay*.

**Standard solution:** 2.7 µg/mL of USP Brompheniramine Maleate RS in *Diluent*, equivalent to 2.0 µg/mL of brompheniramine. Sonicate 1 min.

**Sensitivity solution:** 0.74 µg/mL of USP Pheniramine Maleate RS in *Diluent*

**Sample solution:** 0.5 mg/mL of Brompheniramine Maleate in *Diluent*. Sonicate for 1 min.

**System suitability**

**Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between chlorpheniramine and brompheniramine; and NLT 2.0 between chlorpheniramine related compound B and pheniramine, *System suitability solution*

**Signal-to-noise ratio:** NLT 10 for pheniramine, *Sensitivity solution*

**Relative standard deviation:** NMT 5.0% for brompheniramine, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Brompheniramine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of brompheniramine from the *Standard solution*

$C_S$  = concentration of brompheniramine in the *Standard solution* (mg/mL)

$C_U$  = concentration of Brompheniramine Maleate in the *Sample solution* (mg/mL)

$F$  = relative response factor (see Table 2)

**Acceptance criteria:** See Table 2. Disregard any peak having areas less than 0.05% of brompheniramine.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maleic acid <sup>a</sup>	0.18	—	—
Chlorpheniramine related compound B <sup>b</sup>	0.46	—	—
Pheniramine	0.53	0.45	0.4
Chlorpheniramine	0.94	1.1	0.4
Brompheniramine	1.0	—	—
Any other unspecified impurity	—	1.0	0.10
Total impurities	—	—	1

<sup>a</sup> Salt counter ion is included in the table for identification purposes only.

<sup>b</sup> Di(pyridin-2-yl)amine. Used only to establish the system suitability.

**SPECIFIC TESTS**• **OPTICAL ROTATION** (781)

**Sample:** 100 mg/mL in water at 20°

**Acceptance criteria:** −0.2° to +0.2°, measured in a 20-cm tube



- **pH** (791)  
Sample: 10 mg/mL  
Acceptance criteria: 4.0–5.0
- **Loss on Drying** (731)  
Analysis: Dry at 105° for 3 h.  
Acceptance criteria: NMT 0.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Brompheniramine Maleate RS  
USP Chlorpheniramine Maleate RS  
USP Chlorpheniramine Related Compound B RS  
Di(pyridin-2-yl)amine.  
 $C_{10}H_9N_3$  171.20  
USP Pheniramine Maleate RS

### Brompheniramine Maleate Injection

» Brompheniramine Maleate Injection is a sterile solution of Brompheniramine Maleate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—  
USP Brompheniramine Maleate RS  
USP Endotoxin RS

**Identification**—Dilute a volume of Injection, equivalent to about 50 mg of brompheniramine maleate, with dilute hydrochloric acid (1 in 1200) to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with "Transfer the liquid to a separator;" the Injection meets the requirements of the test.

**Bacterial Endotoxins Test** (85)—It contains not more than 35.7 USP Endotoxin Units per mg of brompheniramine maleate.

**pH** (791): between 6.3 and 7.3.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—Proceed with Injection as directed under *Salts of Organic Nitrogenous Bases* (501), to prepare the solution employed for the determination of the absorbance,  $A_u$ , at 262 nm. For the determination of  $A_s$ , dissolve about 25 mg of USP Brompheniramine Maleate RS, accurately weighed, in 20 mL of dilute sulfuric acid (1 in 350), and treat this solution the same as the portion of Injection being assayed. Calculate the quantity, in mg, of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$  in each mL of the Injection taken by the formula:

$$(W/V)(A_u/A_s)$$

in which  $W$  is the weight, in mg, of USP Brompheniramine Maleate RS in the *Standard Preparation*, and  $V$  is the volume, in mL, of Injection taken.

### Brompheniramine Maleate Oral Solution

» Brompheniramine Maleate Oral Solution contains not less than 95.0 percent and not more

than 105.0 percent of the labeled amount of brompheniramine maleate ( $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ ).

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—  
USP Brompheniramine Maleate RS

**Identification**—Transfer a volume of Oral Solution, equivalent to about 50 mg of brompheniramine maleate, to a separator, render distinctly alkaline with 1 N sodium hydroxide, and extract with two 50-mL portions of chloroform, shaking gently to avoid emulsification. Wash the combined chloroform extracts with 10 mL of water, and discard the aqueous phase. Filter the combined chloroform extracts into a conical flask, and evaporate the solvent on a steam bath, with the aid of a current of air. To the residue add 25 mL of dilute hydrochloric acid (1 in 1200), and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with "Transfer the liquid to a separator." The Oral Solution meets the requirements of the test.

**pH** (791): between 2.5 and 3.5.

**Alcohol Determination, Method I** (611): between 2.7% and 3.3% of  $C_2H_5OH$ .

**Assay**—Transfer an accurately measured volume of Oral Solution, equivalent to about 20 mg of brompheniramine maleate, to a separator, render distinctly alkaline with 1 N sodium hydroxide, and extract with ten 10-mL portions of chloroform, shaking gently to avoid emulsification. Wash the combined chloroform extracts with 10 mL of water, wash the latter with 20 mL of chloroform, and discard the aqueous phase. Quantitatively filter the combined chloroform extracts and washings into a conical flask, and evaporate the solvent on a steam bath, with the aid of a current of air. To the residue add 25 mL of glacial acetic acid and 5 mL of acetic anhydride, agitate, and allow to stand for about 15 minutes. Add 1 drop of crystal violet TS, and titrate with 0.01 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.01 N perchloric acid is equivalent to 2.177 mg of brompheniramine maleate ( $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ ).

### Brompheniramine Maleate Tablets

» Brompheniramine Maleate Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—  
USP Brompheniramine Maleate RS

**Identification**—Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* (181).

**Dissolution** (711)—

Medium: water; 500 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

**Procedure**—Determine the amount of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 264 nm on filtered portions of the solution under test, suitably diluted with 3 N hydrochloric acid, using 5-cm cuvettes, in comparison with a Standard solution having a known concentration of USP Brompheniramine Maleate RS in the same Medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$  is dissolved in 45 minutes.



**Uniformity of dosage units (905):** meet the requirements.

**Assay—**

**Standard preparation—**Dissolve an accurately weighed quantity of USP Brompheniramine Maleate RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 160 µg per mL. Transfer 25.0 mL of this solution to a separator containing 25 mL of water, mix, and proceed as directed under *Assay preparation*, beginning with "adjust with sodium hydroxide solution (1 in 10) to a pH of 11." The concentration of USP Brompheniramine Maleate RS in the *Standard preparation* is about 20 µg per mL.

**Assay preparation—**Weigh and finely powder not fewer than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of brompheniramine maleate, mix with 50 mL of water for 10 minutes, adjust with sodium hydroxide solution (1 in 10) to a pH of 11, and cool to room temperature. Extract the mixture with two 75-mL portions of solvent hexane, and combine the extracts in a second separator. Extract the solvent hexane solution with three 50-mL portions of dilute hydrochloric acid (1 in 120), combining the acid extracts in a 200-mL volumetric flask. Add dilute hydrochloric acid (1 in 120) to volume, and mix.

**Procedure—**Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation*, in 1-cm cells at the wavelength of maximum absorbance at about 264 nm, with a suitable spectrophotometer, using dilute hydrochloric acid (1 in 120) as the blank. Calculate the quantity, in mg, of  $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$  in the portion of Tablets taken by the formula:

$$0.2C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Brompheniramine Maleate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Brompheniramine Maleate and Pseudoephedrine Sulfate Oral Solution

» Brompheniramine Maleate and Pseudoephedrine Sulfate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of brompheniramine maleate ( $C_{16}H_{19}BrN_2 \cdot C_4H_4O_4$ ) and pseudoephedrine sulfate [ $(C_{10}H_{15}NO)_2 \cdot H_2SO_4$ ].

**USP Reference standards (11)—**

USP Brompheniramine Maleate RS  
USP Pseudoephedrine Sulfate RS

**Identification—**

**A:** The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** A solution of it meets the requirements of the test for *Sulfate* (191).

**C:** Transfer a volume of Oral Solution, equivalent to about 6 mg of brompheniramine maleate, to a separator, add 0.5 mL of ammonium hydroxide and 5 mL of methylene chloride, shake for 1 minute, and allow the layers to separate. Use the clear, lower layer as the test solution. Prepare separate *Standard* solutions in methanol containing, respectively, 1.2 mg of USP Brompheniramine Maleate RS and 9 mg of USP Pseudoephedrine Sulfate RS per mL. Separately apply 5 µL of each solution to a suitable thin-layer chromat-

ographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl ether, methanol, and ammonium hydroxide (16:3:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the  $R_f$  values of the two principal spots obtained from the test solution correspond to those obtained from the *Standard* solutions.

**Uniformity of dosage units (905)—**

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume (698)—**

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**Assay—**

**Mobile phase—**Prepare a mixture of water, acetonitrile, methanol, and tetrahydrofuran (550:320:80:50). Transfer 1.0 mL of phosphoric acid, followed by 4.33 g of dodecyl sulfate sodium to this mixture, and mix. Adjust with ammonium hydroxide to a pH of  $3.50 \pm 0.05$ , filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—The pH of the *Mobile phase* is critical and may cause 1 to 4 minutes of differences in the retention times of internal standard and brompheniramine maleate.]

**Internal standard solution—**Transfer about 50 mg of naphazoline hydrochloride to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix.

**Standard preparation—**Dissolve an accurately weighed quantity of USP Brompheniramine Maleate RS in *Mobile phase*, and quantitatively dilute with *Mobile phase* to obtain a solution having a known concentration of about 6000 µg per mL,  $I$  being the ratio of the labeled amount, in mg, of brompheniramine maleate to the labeled amount, in mg, of pseudoephedrine sulfate per mL (*Solution P*). Transfer about 30 mg of USP Pseudoephedrine Sulfate RS, accurately weighed, to a 25-mL volumetric flask, add 5.0 mL each of *Solution P* and *Internal standard solution*, dilute with *Mobile phase* to volume, and mix to obtain a *Standard preparation* having known concentrations of about 1200 µg of USP Brompheniramine Maleate RS per mL and about 1.2 mg of USP Pseudoephedrine Sulfate RS per mL.

**Assay preparation—**Using a "To contain" pipet transfer an accurately measured volume of Oral Solution, equivalent to about 30 mg of pseudoephedrine sulfate, to a 25-mL volumetric flask. Rinse the pipet with about 5 mL of *Mobile phase*, collecting the rinse in the volumetric flask. Add 5.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system (see *Chromatography* (621))—**The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L11. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1.0 for pseudoephedrine sulfate, 1.5 for naphazoline hydrochloride, and 2.5 for brompheniramine maleate; the resolution,  $R$ , between the pseudoephedrine sulfate and naphazoline hydrochloride peaks is not less than 3, and between the brompheniramine maleate and naphazoline hydrochloride peaks is not less than 3; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure—**Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of brompheniramine maleate ( $C_{16}H_{19}BrN_2$  ·

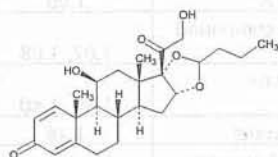


$C_4H_4O_4$ ) in each mL of the Oral Solution taken by the formula:

$$25CV(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Brompheniramine Maleate RS in the *Standard preparation*; V is the volume, in mL, of Oral Solution taken; and  $R_U$  and  $R_S$  are the peak response ratios obtained for brompheniramine maleate and naphazoline hydrochloride from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of pseudoephedrine sulfate ( $C_{10}H_{15}NO_2 \cdot H_2SO_4$ ) in each mL of the Oral Solution taken by the same formula, changing the terms to refer to pseudoephedrine sulfate.

## Budesonide



$C_{25}H_{34}O_6$  430.53  
Pregna-1,4-diene-3,20-dione, 16,17-butyridenebis(oxy)-11,21-dihydroxy-, [11b,16a(R)], and 16a,17-[(S)-butyridenebis(oxy)]-11b,21-dihydroxypregna-1,4-diene-3,20-dione;  
(R,S)-11b,16a,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with butyraldehyde [51333-22-3].  
S epimer [51372-28-2].  
R epimer [51372-29-3].

### DEFINITION

Budesonide is a mixture of two epimeric forms, epimer A (C-22S) and epimer B (C-22R). It contains NLT 40.0% and NMT 51.0% of epimer A, and the sum of both epimers is NLT 98.0% and NMT 102.0% of budesonide ( $C_{25}H_{34}O_6$ ), calculated on the dried basis.

[NOTE—Protect all solutions containing Budesonide from light.]

### IDENTIFICATION

- A. INFRARED ABSORPTION (17K)
- B. ULTRAVIOLET ABSORPTION (197U)

Sample solution: 25 µg/mL

Medium: Methanol

Acceptance criteria: Meets the requirements

### ASSAY

#### PROCEDURE

Buffer: 0.5 mL of glacial acetic acid in 1 L of water.

Adjust with potassium hydroxide to a pH of 3.9.

Mobile phase: Acetonitrile and Buffer (45:55)

Standard solution: 0.06 mg/mL of USP Budesonide RS prepared as follows. Transfer USP Budesonide RS to a suitable volumetric flask, dissolve in acetonitrile equivalent to 30% of the flask volume, and dilute with water to volume.

Sample solution: 0.06 mg/mL of Budesonide prepared as follows. Transfer Budesonide to a suitable volumetric flask, dissolve in acetonitrile equivalent to 30% of the flask volume, and dilute with water to volume.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 25-cm; 3-µm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20 µL

### System suitability

Sample: *Standard solution*

[NOTE—The relative retention time for epimer B is 0.96 with respect to epimer A.]

### Suitability requirements

Resolution: NLT 1.2 between the two budesonide epimer peaks

Relative standard deviation NMT 1.0%, for the sum of the peak areas of the two budesonide epimers

### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of budesonide epimer A ( $C_{25}H_{34}O_6$ ) in the portion of Budesonide taken:

$$\text{Result} = [r_{UA} / (r_{UA} + r_{UB})] \times 100$$

$r_{UA}$  = peak area of epimer A from the *Sample solution*

$r_{UB}$  = peak area of epimer B from the *Sample solution*

Calculate the percentage of budesonide ( $C_{25}H_{34}O_6$ ) in the portion of Budesonide taken:

$$\text{Result} = [(r_{UA} + r_{UB}) / (r_{SA} + r_{SB})] \times (C_S / C_U) \times 100$$

$r_{UA}$  = peak area of epimer A from the *Sample solution*

$r_{UB}$  = peak area of epimer B from the *Sample solution*

$r_{SA}$  = peak area of epimer A from the *Standard solution*

$r_{SB}$  = peak area of epimer B from the *Standard solution*

$C_S$  = concentration of USP Budesonide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Budesonide in the *Sample solution* (mg/mL)

### Acceptance criteria

Epimer A: 40.0%–51.0%

Both epimers: 98.0%–102.0% on the dried basis

### IMPURITIES

#### ORGANIC IMPURITIES

Solution A: 0.5 mL of glacial acetic acid in 1 L of water. Adjust with potassium hydroxide to a pH of 3.9.

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
5	75	25
35	68	32
42	59	41
59	25	75
60	75	25
70	75	25

Diluent: Acetonitrile and water (3:7)

System suitability stock solution A: 0.3 mg/mL of USP Budesonide Related Compound E RS in acetonitrile

System suitability stock solution B: 0.3 mg/mL of USP Budesonide Related Compound G RS in acetonitrile



**System suitability stock solution C:** 0.3 mg/mL of USP Budesonide Related Compound L RS in acetonitrile

**System suitability solution:** 0.6 mg/mL of USP Budesonide RS and 3 µg/mL each of USP Budesonide Related Compound E RS, USP Budesonide Related Compound G RS, and USP Budesonide Related Compound L RS in Diluent prepared as follows. Transfer USP Budesonide RS to a suitable volumetric flask and add suitable quantities of *System suitability stock solution A*, *System suitability stock solution B*, and *System suitability stock solution C*. Dilute with acetonitrile equivalent to 30% of the final volume and dilute with water to volume.

**Standard stock solution:** 0.6 mg/mL of USP Budesonide RS prepared as follows. Transfer USP Budesonide RS to a suitable volumetric flask, dissolve in acetonitrile equivalent to 30% of the flask volume, and dilute with water to volume.

**Sensitivity solution:** 0.3 µg/mL of USP Budesonide RS in Diluent from the *Standard stock solution*

**Standard solution:** 6 µg/mL of USP Budesonide RS in Diluent from the *Standard stock solution*

**Sample solution:** 0.6 mg/mL of Budesonide prepared as follows. Transfer Budesonide to a suitable volumetric flask, dissolve in acetonitrile equivalent to 30% of the flask volume, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 25-cm; 3-µm packing L1

**Temperatures**

**Autosampler:** 4°

**Column:** 50°. [NOTE—The resolution between budesonide related compound E and budesonide related compound L may be improved by lowering the temperature, but to NLT 40°.]

**Flow rate:** 1.5 mL/min

**Injection volume:** 50 µL

#### System suitability

**Samples:** *System suitability solution*, *Sensitivity solution*, and *Standard solution*

[NOTE—The relative retention times of the two epimers of budesonide are 0.96 (epimer B) and 1.00 (epimer A), respectively.]

#### Suitability requirements

**Resolution:** NLT 1.2 between budesonide related compound E and budesonide related compound L and NLT 3.0 between budesonide epimer A and the first epimer of budesonide related compound G, *System suitability solution*

**Tailing factor:** NMT 1.5 for the budesonide epimer B peak, *Standard solution*

**Relative standard deviation:** NMT 5.0%, for the sum of the peak areas of the two budesonide epimers, *Standard solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each impurity in the portion of Budesonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of the sum of budesonide epimers from the *Standard solution*

$C_S$  = concentration of USP Budesonide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Budesonide in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 2. Disregard peaks that are less than 0.05% of the total peak areas of the budesonide epimers. Disregard peaks eluting after 60

min, which, if present, are due to the change in the gradient.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
16 $\alpha$ -Hydroxyprednisolone <sup>a</sup>	0.12	0.2
Budesonide acetaldehyde acetal (epimers) <sup>b</sup>	0.39, 0.40	0.10 <sup>c</sup>
Budesonide D-homo analog <sup>d,e</sup>	0.47	0.10
Desonide <sup>e,f</sup>	0.51	0.10
Budesonide glyoxal (epimers) <sup>g</sup>	0.76, 0.78	0.07 <sup>c</sup>
Budesonide related compound E <sup>h</sup>	0.86	0.10
Budesonide related compound L <sup>i</sup>	0.88	0.2
Budesonide epimer B	0.96	—
Budesonide epimer A	1.00	—
Budesonide related compound G (epimers) <sup>j</sup>	1.07, 1.08	0.10 <sup>c</sup>
Budesonide 21-acetate (epimers) <sup>k</sup>	1.39, 1.40	0.10 <sup>c</sup>
Budesonide 21-butyrate <sup>l</sup>	1.48	0.10
Any other individual impurity	—	0.10
Total specified impurities	—	0.4
Total unspecified impurities	—	0.4

<sup>a</sup> 11 $\beta$ ,16 $\alpha$ ,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione.

<sup>b</sup> 16 $\alpha$ ,17-[Ethylidenebis(oxy)]-11 $\beta$ ,21-dihydroxypregna-1,4-diene-3,20-dione.

<sup>c</sup> Limit includes both epimers.

<sup>d</sup> 16 $\alpha$ ,17-[Butylidenebis(oxy)]-11 $\beta$ -hydroxy-17-(hydroxymethyl)-D-homoandrosta-1,4-diene-3,17a-dione; also known as D-homobudesonide.

<sup>e</sup> This impurity is to be reported under total unspecified impurities. Do not report it under total specified impurities.

<sup>f</sup> 16 $\alpha$ ,17-[1-Methylethylidenebis(oxy)]-11 $\beta$ , 21-dihydroxypregna-1,4-diene-3,20-dione.

<sup>g</sup> 16 $\alpha$ ,17-[Butylidenebis(oxy)]-11 $\beta$ -hydroxy-3,20-dioxopregna-1,4-dien-21-al; also known as 21-dehydrobudesonide.

<sup>h</sup> Also known as 14,15-dehydrobudesonide or budesonide 14-ene.

<sup>i</sup> Also known as 11-ketobudesonide.

<sup>j</sup> Also known as 1,2-dihydrobudesonide.

<sup>k</sup> 16 $\alpha$ ,17-[Butylidenebis(oxy)]-11 $\beta$ ,21-dihydroxypregna-1,4-diene-3,20-dione-21-acetate.

<sup>l</sup> 16 $\alpha$ ,17-[Butylidenebis(oxy)]-11 $\beta$ ,21-dihydroxypregna-1,4-diene-3,20-dione-21-butyrate.

#### SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count is NMT 10<sup>3</sup> cfu/g, and the total combined molds and yeast count is NMT 10<sup>2</sup> cfu/g.

• **LOSS ON DRYING** (731)

**Analysis:** Dry at 105° to constant weight.

**Acceptance criteria:** NMT 0.3%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Budesonide RS

USP Budesonide Related Compound E RS

16 $\alpha$ ,17-[Butylidenebis(oxy)]-11 $\beta$ ,21-dihydroxypregna-1,4,14-triene-3,20-dione.

C<sub>25</sub>H<sub>32</sub>O<sub>6</sub> 428.52

USP Budesonide Related Compound G RS

16 $\alpha$ ,17-[Butylidenebis(oxy)]-11 $\beta$ ,21-dihydroxypregna-4-ene-3,20-dione.

C<sub>25</sub>H<sub>36</sub>O<sub>6</sub> 432.55

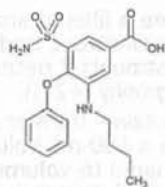
USP Budesonide Related Compound L RS

16 $\alpha$ ,17-[Butylidenebis(oxy)]-21-hydroxypregna-1,4-diene-3,11,20-trione.



C<sub>25</sub>H<sub>32</sub>O<sub>6</sub> 428.52

## Bumetanide



C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>S 364.42  
Benzoic acid, 3-(aminosulfonyl)-5-(butylamino)-4-phenoxy-;  
3-(Butylamino)-4-phenoxy-5-sulfamoylbenzoic acid  
[28395-03-1].

### DEFINITION

Bumetanide contains NLT 98.0% and NMT 102.0% of bumetanide (C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>S), calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION (197M)**
- B. ULTRAVIOLET ABSORPTION (197U)**  
Sample solution: 50 µg/mL in isopropyl alcohol  
Acceptance criteria: Meets the requirements
- C.** The principal spot of the *Sample solution* exhibits an *R<sub>f</sub>* value corresponding to that of *Standard solution A*, as obtained in the test for *Organic Impurities*.

### ASSAY

- PROCEDURE**  
Sample solution: Dissolve 1 g of Bumetanide in 150 mL of alcohol, and add phenol red TS.  
Titrimetric system  
Mode: Direct titration  
Titrant: 0.1 N sodium hydroxide VS  
Analysis: Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N sodium hydroxide is equivalent to 36.44 mg of bumetanide (C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>S.)  
Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

- RESIDUE ON IGNITION (281):** NMT 0.1%, on a 1-g specimen

### Delete the following:

- HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-

Jan-2018)

### ORGANIC IMPURITIES

Standard solution A: 25 mg/mL of USP Bumetanide RS in methanol  
Standard solution B: 50 µg/mL of USP Bumetanide RS from *Standard solution A* in methanol  
Standard solution C: 50 µg/mL of USP Bumetanide Related Compound B RS in methanol  
Standard solution D: 25 µg/mL of USP Bumetanide Related Compound A RS in methanol  
Standard solution E: 25 µg/mL of USP Butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate RS in methanol  
Sample solution: 25 mg/mL of Bumetanide in methanol  
Chromatographic system  
(See *Chromatography* (621), *Thin-Layer Chromatography*.)  
Mode: TLC  
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

**Developing solvent system:** Chloroform, cyclohexane, glacial acetic acid, and methanol (160:20:20:5)

**Application volume:** 20 µL

### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, *Standard solution D*, *Standard solution E*, and *Sample solution*

Proceed as directed in *Chromatography* (621). After drying the application spots, place the plate in an unlined and unsaturated chromatographic chamber.

Examine the plate under short-wavelength UV light.

**Acceptance criteria:** See *Table 1*. Any secondary spots from the *Sample solution* are not larger or more intense than the corresponding principal spots from the corresponding standard solution identified in *Table 1*.

Table 1

Name	Corresponding Standard Solution	Acceptance Criteria, NMT (%)
Bumetanide related compound A <sup>a</sup>	Standard solution D	0.1
Bumetanide related compound B <sup>a</sup>	Standard solution C	0.2
Butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate	Standard solution E	0.1
Other individual impurities	Standard solution B	0.2
Sum of other individual impurities <sup>a</sup>	—	0.4

<sup>a</sup> Excluding bumetanide related compound A, bumetanide related compound B, and butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate.

### SPECIFIC TESTS

- LOSS ON DRYING (731)**  
Sample: Dry a sample at 105° for 4 h.  
Acceptance criteria: NMT 0.5%

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**  
USP Bumetanide RS  
USP Bumetanide Related Compound A RS  
3-Amino-4-phenoxy-5-sulfamoylbenzoic acid.  
C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>5</sub>S 308.31  
USP Bumetanide Related Compound B RS  
3-Nitro-4-phenoxy-5-sulfamoylbenzoic acid.  
C<sub>13</sub>H<sub>10</sub>N<sub>2</sub>O<sub>7</sub>S 338.29  
USP Butyl 3-(butylamino)-4-phenoxy-5-sulfamoylbenzoate RS  
C<sub>21</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub>S 420.53

## Bumetanide Injection

» Bumetanide Injection is a sterile solution of Bumetanide in Water for Injection, prepared with the aid of Sodium Hydroxide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of bumetanide (C<sub>17</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>S).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

### USP Reference standards (11)—

USP Bumetanide RS



USP Bumetanide Related Compound A RS  
3-Amino-4-phenoxy-5-sulfamoylbenzoic acid.  
 $C_{13}H_{12}N_2O_5S$  308.31

USP Endotoxin RS

#### Identification—

**A:** The relative retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**B:** The principal spot obtained from the chromatogram of the *Test solution* exhibits an  $R_f$  value corresponding to that of the *Identification solution*, as obtained in the test for *Related compounds*.

**Bacterial Endotoxins Test** (85)—It contains not more than 350 USP Endotoxin Units per mg of bumetanide.

**pH** (791): between 6.8 and 7.8.

#### Related compounds—

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Test solution**—Pipet a volume of *Injection*, equivalent to 5 mg of bumetanide, into a 125-mL separator, and adjust with 0.1 N sodium hydroxide to a pH of 12. Extract with two 20-mL portions of ethyl ether, discard the ethyl ether extracts, and adjust the aqueous layer with 1 N acetic acid to a pH of 4. Extract with two 20-mL portions of ethyl ether, passing the extracts through anhydrous sodium sulfate. Wash the sodium sulfate with about 5 mL of ethyl ether. Evaporate the combined ethyl ether extracts with the aid of a stream of nitrogen to dryness, and dissolve the residue in 0.5 mL of methanol.

**Identification solution**—Dissolve USP Bumetanide RS in methanol to obtain a solution having a concentration of about 10 mg per mL.

**Standard solutions**—Dilute a volume of the *Identification solution* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.08 mg of USP Bumetanide RS per mL. Quantitatively dilute with methanol to obtain *Standard solutions* having the following compositions.

Standard solution	Dilution	Concentration ( $\mu$ g of RS per mL)	Percentage (% for comparison with test specimen)
1	undiluted	80	0.8
2	3 in 4	60	0.6
3	1 in 2	40	0.4
4	1 in 4	20	0.2
5	1 in 8	10	0.1

**Standard solution 6**—Dissolve an accurately weighed quantity of USP Bumetanide Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.02 mg per mL.

**Application volume:** 50  $\mu$ L.

**Developing solvent system:** a mixture of chloroform, cyclohexane, glacial acetic acid, and methanol (80:10:10:2.5).

**Procedure**—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Examine the plate under short-wavelength UV light. Any secondary spot obtained from the chromatogram of the *Test solution* having an  $R_f$  value corresponding to the  $R_f$  value of the principal spot obtained from the chromatogram of *Standard solution 6* is not larger or more intense than the principal spot obtained from the chromatogram of *Standard solution 6*: not more than 0.2% of bumetanide related compound A is found. For all other secondary spots obtained from the chromatogram of the *Test solution*, compare the intensity of each spot with the principal spots obtained from the chromatograms of

*Standard solutions 1 through 5*: not more than 0.2% of any individual other impurity is found; and not more than 0.8% of the sum of all other impurities is found (excluding bumetanide related compound A).

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, water, tetrahydrofuran, and glacial acetic acid (50:45:5:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer about 50 mg of 4-ethylbenzaldehyde to a 100-mL volumetric flask. Dissolve in and dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 100-mL volumetric flask, add 10.0 mL of tetrahydrofuran and 4.0 mL of glacial acetic acid, dilute with methanol to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Bumetanide RS in *Internal standard solution*, and quantitatively dilute with *Internal standard solution* to obtain a solution having a known concentration of about 250  $\mu$ g per mL. Transfer 5.0 mL of the resulting solution to a 10-mL volumetric flask, dilute with water to volume, and mix to obtain a solution having a known concentration of about 125  $\mu$ g of USP Bumetanide RS per mL.

**Assay preparation**—Transfer an accurately measured volume of *Injection*, equivalent to about 0.25 mg of bumetanide, to a flask. Add an equal volume of *Internal standard solution*, accurately measured, insert the stopper, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for 4-ethylbenzaldehyde and 1.0 for bumetanide; the resolution,  $R$ , between the analyte and internal standard peaks is not less than 1.5, the tailing factor for the analyte peak is not more than 1.4, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{17}H_{20}N_2O_5S$  in each mL of the *Injection* taken by the formula:

$$(2C/V)(R_U/R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Bumetanide RS in the *Standard preparation*;  $V$  is the volume, in mL, of *Injection* taken; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Bumetanide Tablets

» Bumetanide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of bumetanide ( $C_{17}H_{20}N_2O_5S$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Bumetanide RS

USP Bumetanide Related Compound A RS

3-Amino-4-phenoxy-5-sulfamoylbenzoic acid.

$C_{13}H_{12}N_2O_5S$  308.31



**Identification—**

**A:** The relative retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** The principal spot obtained from the chromatogram of the *Test solution* exhibits an  $R_f$  value corresponding to that of the *Identification solution*, as obtained in the test for *Related compounds*.

**Dissolution (711)—**

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**pH 2.9 Glycine buffer—**Dissolve 7.505 g of glycine and 5.85 g of sodium chloride in water to make 1000 mL (stock solution). Dilute 80.0 mL of the stock solution and 20.0 mL of 0.1 N hydrochloric acid with water to 1000 mL. Adjust, if necessary, with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 2.9.

**Procedure—**Determine the amount of  $C_{17}H_{20}N_2O_5S$  dissolved, by employing a suitable fluorometer having an excitation wavelength of about 350 nm and a fluorescence emission of about 450 nm on filtered portions of the solution under test, suitably diluted with pH 2.9 Glycine buffer, in comparison with a Standard solution having a known concentration of USP Bumetanide RS in the same Medium.

**Tolerances—**Not less than 85% (Q) of the labeled amount of  $C_{17}H_{20}N_2O_5S$  is dissolved in 30 minutes.

**Uniformity of dosage units (905):** meet the requirements.

**Related compounds—**

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Test solution—**Transfer an accurately weighed portion of finely powdered Tablets, equivalent to 10 mg of bumetanide, to a 50-mL centrifuge tube, add 20 mL of acetone (spectrophotometric or HPLC quality), and shake by mechanical means for 10 minutes. Centrifuge for 10 minutes, decant the supernatant into a glass-stoppered, 25-mL conical flask, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 0.5 mL of methanol.

**Identification solution—**Dissolve USP Bumetanide RS in methanol to obtain a solution having a concentration of about 20 mg per mL.

**Standard solutions—**Dilute a volume of the *Identification solution* quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.16 mg of USP Bumetanide RS per mL. Quantitatively dilute with methanol to obtain *Standard solutions* having the following compositions.

Standard solution	Dilution	Concentration (µg of RS per mL)	Percentage (% for comparison with test specimen)
1	undiluted	160	0.8
2	3 in 4	120	0.6
3	1 in 2	80	0.4
4	1 in 4	40	0.2
5	1 in 8	20	0.1

**Standard solution 6—**Dissolve an accurately weighed quantity of USP Bumetanide Related Compound A RS in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain a solution having a known concentration of about 0.04 mg per mL.

**Application volume:** 25 µL.

**Developing solvent system:** a mixture of chloroform, cyclohexane, glacial acetic acid, and methanol (80:10:10:2.5).

**Procedure—**Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Examine the plate under short-wavelength UV light. Any secondary spot obtained from the chromatogram of the *Test solution* having an  $R_f$  value corresponding to the  $R_f$  value of the principal spot obtained from the chromatogram of *Standard solution 6* is not larger or more intense than the principal spot obtained from the chromatogram of *Standard solution 6*; not more than 0.2% of bumetanide related compound A is found. For all other secondary spots obtained from the chromatogram of the *Test solution*, compare the intensity of each spot with the principal spots obtained from the chromatograms of *Standard solutions 1* through *5*; not more than 0.2% of any individual other impurity is found; and not more than 0.8% of the sum of all other impurities is found (excluding bumetanide related compound A).

**Assay—**

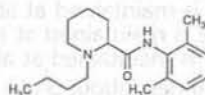
**Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system—**Prepare as directed in the *Assay* under *Bumetanide Injection*.

**Assay preparation—**Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.5 mg of bumetanide, to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution*, and sonicate for 5 minutes. Add 2.0 mL of water, and mix. Cool, and filter, discarding the first 1 mL of the filtrate.

**Procedure—**Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.7 for 4-ethylbenzaldehyde and 1.0 for bumetanide. Calculate the quantity, in mg, of bumetanide ( $C_{17}H_{20}N_2O_5S$ ) in the portion of Tablets taken by the formula:

$$4C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Bumetanide RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Bupivacaine Hydrochloride**

• HCl • H<sub>2</sub>O

$C_{18}H_{28}N_2O \cdot HCl \cdot H_2O$  342.90

2-Piperidinecarboxamide, 1-butyl-N-(2,6-dimethylphenyl)-, monohydrochloride, monohydrate, (±)-.

(±)-1-Butyl-2',6'-pipecoloxylidide monohydrochloride, monohydrate [73360-54-0].

Anhydrous 324.90 [18010-40-7].

» Bupivacaine Hydrochloride contains not less than 98.5 percent and not more than 101.5 percent of  $C_{18}H_{28}N_2O \cdot HCl$ , calculated on the anhydrous basis.

**Packaging and storage—**Preserve in well-closed containers.

**USP Reference standards (11)—**

USP Bupivacaine Hydrochloride RS



**Identification—****A: Infrared Absorption** (197S)—

**Solution**—Dissolve about 230 mg in 15 mL of water in a separator, add 1 mL of 6 N ammonium hydroxide, and extract with three 30-mL portions of chloroform. Evaporate the chloroform at room temperature with the aid of a stream of nitrogen, and dry the residue in vacuum. Add 2 mL of chloroform to the residue, and dissolve.

**B: Ultraviolet Absorption** (197U)—**Solution:** 500 µg per mL.**Medium:** 0.1 N hydrochloric acid.

Absorptivities at 271 nm, calculated on the anhydrous basis, do not differ by more than 3.0%.

**C:** Dissolve about 50 mg in 10 mL of water in a small separator, render alkaline with 6 N ammonium hydroxide, and extract with 10 mL of ether: the aqueous layer meets the requirements of the tests for *Chloride* (191).

**pH** (791): between 4.5 and 6.0, in a solution (1 in 100).**Water Determination, Method I** (921): between 4.0% and 6.0%.**Residue on ignition** (281): not more than 0.1%.**Delete the following:**

• **Heavy metals, Method II** (231): not more than 0.001%. • (Official 1-Jan-2018)

**Limit of residual solvents—**

**Alcohol standard solution**—Pipet 2 mL of dehydrated alcohol into a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix. The resulting solution contains 0.08% of alcohol.

**Isopropyl alcohol standard solution**—Pipet 2 mL of isopropyl alcohol into a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. The resulting solution contains 0.004% of isopropyl alcohol.

**Test solution**—Transfer 1.0 g of Bupivacaine Hydrochloride, accurately weighed, to a 25-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system**—Under typical conditions, the instrument is equipped with a flame-ionization detector and a 4-mm × 2-m column that contains packing S3. The carrier gas is nitrogen, flowing at a rate of about 40 mL per minute. The column temperature is maintained at about 175°, the injection port temperature is maintained at about 200°, and the detector temperature is maintained at about 280°.

**Procedure**—Inject equal volumes (about 5 µL) of the *Test solution*, the *Alcohol standard solution*, and the *Isopropyl alcohol standard solution* successively into the gas chromatograph. Measure the responses of the alcohol peak and the isopropyl alcohol peak in each chromatogram. Determine the percentage of alcohol taken by the formula:

$$2(r_U / r_S)$$

and determine the percentage of isopropyl alcohol taken by the formula:

$$0.1(r_U / r_S)$$

in which  $r_U$  and  $r_S$  are the responses of the respective analytes in the *Test solution* and of the corresponding analytes in the *Alcohol standard solution* and the *Isopropyl alcohol standard solution*, respectively. The sum of the content of alcohol and the content of isopropyl alcohol does not exceed 2%.

**Chromatographic purity—**

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture.

**Solvent:** a mixture of chloroform and isopropylamine (99:1).

**Test solution**—Dissolve a suitable quantity of Bupivacaine Hydrochloride in *Solvent* to obtain a solution containing 20.0 mg per mL.

**Standard solution**—Dissolve a suitable quantity of USP Bupivacaine Hydrochloride RS, accurately weighed, in *Solvent* to obtain a solution containing 20.0 mg per mL.

**Diluted standard solution**—Quantitatively dilute a portion of the *Standard solution* in *Solvent* to obtain a solution having a concentration of 100 µg per mL.

**Developing solvent system:** a mixture of hexanes and isopropylamine (97:3).

**Procedure**—Apply separate 10-µL portions of the *Test Solution*, the *Standard solution*, and the *Diluted standard solution* on the starting line of suitable thin-layer chromatographic plate as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Develop the chromatogram in a suitable chamber until the solvent has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and dry it in warm air. Place the plate in a closed chamber with a dish containing 1 g of iodine in a shallow layer, and allow to remain for about 5 minutes. Remove the plate from the chamber, spray it with 7 N sulfuric acid, and examine the chromatogram: the  $R_f$  value of the principal spot from the *Test solution* corresponds to that of the *Standard solution*, and the estimated size and intensity of any other spot obtained from the *Test solution* does not exceed that of the principal spot obtained from the *Diluted standard solution* (0.5%); and the total of the estimated sizes and intensities of all of the other spots obtained from the *Test solution* does not exceed four times that of the principal spot obtained from the *Diluted standard solution* (2.0%).

**Assay**—Transfer about 600 mg of Bupivacaine Hydrochloride, accurately weighed, to a 250-mL conical flask, and dissolve in 20 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS and 3 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 32.49 mg of  $C_{18}H_{28}N_2O \cdot HCl$ .

**Bupivacaine Hydrochloride Injection****DEFINITION**

Bupivacaine Hydrochloride Injection is a sterile solution of Bupivacaine Hydrochloride in Water for Injection. It contains NLT 93.0% and NMT 107.0% of the labeled amount of bupivacaine hydrochloride ( $C_{18}H_{28}N_2O \cdot HCl$ ).

**IDENTIFICATION**• **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES** (181)

**Sample solution:** 2 mg/mL of bupivacaine hydrochloride in 0.01 N hydrochloric acid, from Injection

**Analysis:** Proceed as directed in the chapter beginning with "Transfer the liquid to a separator".

**Acceptance criteria:** Meets the requirements

- **B.** The retention time of the bupivacaine peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Buffer:** 1.94 g/L of monobasic potassium phosphate and 2.48 g/L of dibasic potassium phosphate in water.



Adjust, if necessary, with 1 N potassium hydroxide or 1 M phosphoric acid to a pH of 6.8.

**Mobile phase:** Acetonitrile and Buffer (65:35). Adjust, if necessary, with 1 M phosphoric acid to a pH of  $7.7 \pm 0.2$ . Filter the solution through a membrane filter of 1- $\mu$ m or finer pore size, and degas.

**Internal standard solution:** 1.3 mg/mL of dibutyl phthalate in methanol

**Standard solution:** 0.5 mg/mL of USP Bupivacaine Hydrochloride RS, prepared as follows. In a 100-mL volumetric flask, dissolve 50 mg of USP Bupivacaine Hydrochloride RS in 10.0 mL of water, using sonication if necessary. Add 10 mL of *Internal standard solution*, and dilute with methanol to volume.

**Sample solution:** Nominally 0.5 mg/mL of bupivacaine hydrochloride, prepared as follows. In a 100-mL volumetric flask, transfer an amount of Injection equivalent to 50 mg of bupivacaine hydrochloride, add 10.0 mL of *Internal standard solution*, and dilute with methanol to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 263 nm

**Column:** 4-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for bupivacaine hydrochloride and dibutyl phthalate are about 1.0 and 1.2, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between bupivacaine hydrochloride and dibutyl phthalate

**Relative standard deviation:** NMT 1.0% for the ratio of bupivacaine to the internal standard from three replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bupivacaine hydrochloride ( $C_{18}H_{28}N_2O \cdot HCl$ ) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of bupivacaine to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of bupivacaine to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Bupivacaine Hydrochloride RS, calculated on the anhydrous basis, in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bupivacaine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–107.0%

#### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 2.5 USP Endotoxin Units/mg of bupivacaine hydrochloride
- **pH** (791): 4.0–6.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or in multiple-dose containers, preferably of Type I glass. Injection labeled to contain 0.5% or less of bupivacaine hydrochloride may be packaged in 50-mL, multiple-dose containers.

#### • USP REFERENCE STANDARDS (11)

USP Bupivacaine Hydrochloride RS  
USP Endotoxin RS

### Bupivacaine Hydrochloride in Dextrose Injection

» Bupivacaine Hydrochloride in Dextrose Injection is a sterile solution of Bupivacaine Hydrochloride and Dextrose in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amounts of bupivacaine hydrochloride ( $C_{18}H_{28}N_2O \cdot HCl$ ) and dextrose ( $C_6H_{12}O_6$ ). It contains no preservative.

**Packaging and storage:**—Preserve in single-dose containers, preferably of Type I glass.

#### USP Reference standards (11)—

USP Bupivacaine Hydrochloride RS

USP Dextrose RS

USP Endotoxin RS

#### Identification—

**A:** *Thin-Layer Chromatographic Identification Test* (201)—

**Adsorbent:** chromatographic silica gel mixture; 0.25 mm.

**Developing solvent:** mixture of butyl alcohol, water, dehydrated alcohol, and glacial acetic acid (6:2:1:1).

**Test preparation:** Bupivacaine Hydrochloride in Dextrose Injection.

**Standard preparations A, B, and C:**—Separately prepare (A) a solution of USP Bupivacaine Hydrochloride RS in water, (B) a solution of USP Dextrose RS in water, and (C) a solution of USP Bupivacaine Hydrochloride RS in (B) to obtain solutions having concentrations corresponding to the labeled concentrations of bupivacaine hydrochloride and dextrose in the Injection.

**Naphthalenediol reagent:**—Dissolve 20 mg of 1,3-naphthalenediol in 10 mL of dehydrated alcohol containing 0.2 mL of sulfuric acid.

**Iodoplatinate reagent:**—Mix equal volumes of platinum chloride solution (3 in 1000) and potassium iodide solution (6 in 100).

**Procedure:**—Separately apply 10  $\mu$ L each of the *Test preparation* and *Standard preparations A* and *C* to a portion of the chromatographic plate, and separately apply 1  $\mu$ L each of the *Test preparation* and *Standard preparation B* to the remaining portion of the plate. Dry the applications in a current of warm air, develop the chromatograms, remove the plate from the developing chamber, and mark the solvent front. Dry the plate in warm circulating air, and examine the plate under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the *Test preparation* corresponds to the spots obtained from the adjacent chromatograms of *Standard preparations A* and *C*. Spray the plate with *Naphthalenediol reagent*, heat at 90° for 5 minutes, and examine the plate: the  $R_f$  value of the principal blue-purple spot obtained from the *Test preparation* corresponds to that obtained in the adjacent chromatogram of *Standard preparation B*. Cool the plate, spray it with *Iodoplatinate reagent*, and examine the plate: bupivacaine appears as a blue-purple spot on a salmon-colored background, and the dextrose spots fade slightly: the  $R_f$  value of the bupivacaine spot obtained from the *Test preparation* corresponds to those obtained from the adjacent chromatograms of *Standard preparations A* and *C*.

**B:** It responds to *Identification test B* under *Bupivacaine Hydrochloride Injection*.



**Bacterial Endotoxins Test** (85)—It contains not more than 1.8 USP Endotoxin Units per mg of bupivacaine hydrochloride.

**pH** (791): between 4.0 and 6.5.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay for bupivacaine hydrochloride—**

*pH* 6.8 Phosphate buffer, *Mobile phase*, *Internal standard solution*, *Standard preparation*, *Chromatographic system*, and *Procedure*—Proceed as directed in the Assay under *Bupivacaine Hydrochloride Injection*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of bupivacaine hydrochloride, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

**Assay for dextrose**—Determine the angular rotation of Injection in a suitable polarimeter tube (see *Optical Rotation* (781)). Calculate the percentage (g per 100 mL) of dextrose ( $C_6H_{12}O_6$ ) in the portion of Injection taken by the formula:

$$(100/52.9)AR$$

in which 100 is the percentage; 52.9 is the midpoint of the specific rotation range for anhydrous dextrose, in degrees;  $A$  is 100 mm divided by the length of the polarimeter tube, in mm; and  $R$  is the observed rotation, in degrees.

## Bupivacaine Hydrochloride and Epinephrine Injection

» Bupivacaine Hydrochloride and Epinephrine Injection is a sterile solution of Bupivacaine Hydrochloride and Epinephrine or Epinephrine Bitartrate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of bupivacaine hydrochloride ( $C_{18}H_{28}N_2O \cdot HCl$ ). The content of epinephrine ( $C_9H_{13}NO_3$ ) does not exceed 0.001 percent (1 in 100,000). It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of epinephrine ( $C_9H_{13}NO_3$ ).

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light. Injection labeled to contain 0.5% or less of bupivacaine hydrochloride may be packaged in 50-mL multiple-dose containers.

**Labeling**—The label indicates that the Injection is not to be used if its color is pinkish or darker than slightly yellow or if it contains a precipitate.

**USP Reference standards** (11)—

USP Bupivacaine Hydrochloride RS

USP Epinephrine Bitartrate RS

USP Endotoxin RS

**Color and clarity**—Using the Injection as the *Test solution*, proceed as directed for *Color and clarity* under *Epinephrine Injection*.

**Identification—**

**A:** It responds to the *Identification* tests under *Bupivacaine Hydrochloride Injection*.

**B:** Pipet a volume of Injection, equivalent to about 50  $\mu$ g of epinephrine, into a suitable container, add 0.1 mL of

*Ferro-citrate solution* and 2.0 mL of *Buffer solution* (prepared as directed under *Epinephrine Assay* (391)), mix, and allow the solution to stand for 10 minutes. Filter the solution: the filtrate is violet in color and may turn brownish.

**Bacterial Endotoxins Test** (85)—It contains not more than 1.6 USP Endotoxin Units per mg of bupivacaine hydrochloride.

**pH** (791): between 3.3 and 5.5.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay for bupivacaine hydrochloride—**

*pH* 6.8 Phosphate buffer, *Mobile phase*, *Internal standard solution*, *Standard preparation*, *Chromatographic system*, and *Procedure*—Proceed as directed in the Assay under *Bupivacaine Hydrochloride Injection*.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 50 mg of bupivacaine hydrochloride, to a 100-mL volumetric flask, add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

**Assay for epinephrine—**

*Mobile phase*—Prepare a suitably filtered and degassed mixture of water, methanol, and 2M monobasic sodium phosphate (900:50:50), containing in each 1000 mL, 40 mg of edetate disodium, 0.4 mL of phosphoric acid, and 0.4 g of sodium 1-octanesulfonate. Make adjustments, if necessary, to obtain a retention time of not less than 11 minutes for the epinephrine peak (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Epinephrine Bitartrate RS in *Mobile phase* to obtain a solution having a concentration of about 2  $\mu$ g per mL.

*Resolution solution*—Dissolve suitable quantities of epinephrine bitartrate and dopamine hydrochloride in *Mobile phase* to obtain a solution containing about 2  $\mu$ g of each per mL.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 25  $\mu$ g of epinephrine, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with an electrochemical detector held at a potential of +0.75 volt and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the epinephrine and dopamine peaks is not less than 6.0; and the relative retention times are about 2 for dopamine and 1.0 for epinephrine. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

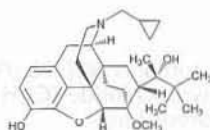
*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of epinephrine ( $C_9H_{13}NO_3$ ) in each mL of the Injection taken by the formula:

$$(183.21 / 333.30)(25)(C / V)(r_u / r_s)$$

in which 183.21 and 333.30 are the molecular weights of epinephrine and epinephrine bitartrate, respectively;  $C$  is the concentration, in  $\mu$ g per mL, of USP Epinephrine Bitartrate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injection taken; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Buprenorphine Hydrochloride



$C_{29}H_{41}NO_4 \cdot HCl$  504.10

6,14-Ethenomorphinan-7-methanol, 17-(cyclopropylmethyl)- $\alpha$ -(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy- $\alpha$ -methyl-, hydrochloride, [5 $\alpha$ ,7 $\alpha$ (5)]-;  
21-Cyclopropyl-7 $\alpha$ -[(5)-1-hydroxy-1,2,2-trimethylpropyl]-6,14-endo-ethano-6,7,8,14-tetrahydrooripavine hydrochloride [53152-21-9].

### DEFINITION

Buprenorphine Hydrochloride contains NLT 98.5% and NMT 101.0% of buprenorphine hydrochloride ( $C_{29}H_{41}NO_4 \cdot HCl$ ), calculated on the anhydrous basis.

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197K)

##### B.

**Sample solution:** 50 mg/mL of Buprenorphine Hydrochloride in methanol

**Analysis:** To 0.5 mL of the *Sample solution* add 0.2 mL of a freshly prepared 100-mg/mL potassium ferricyanide TS solution and 0.5 mL of ferric chloride TS.

**Acceptance criteria:** A blue color appears immediately.

#### C. IDENTIFICATION TESTS—GENERAL, Chloride (191)

**Sample solution:** 10 mg/mL

**Acceptance criteria:** Meets the requirements

### ASSAY

#### PROCEDURE

**Sample:** 0.8 g

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 50 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS and 2 drops of crystal violet TS, and titrate with *Titrant* to a green endpoint. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of *Titrant* is equivalent to 50.41 mg of buprenorphine hydrochloride ( $C_{29}H_{41}NO_4 \cdot HCl$ ).

**Acceptance criteria:** 98.5%–101.0% on the anhydrous basis

### IMPURITIES

#### RESIDUE ON IGNITION (281): NMT 0.1%

#### ORGANIC IMPURITIES

**Mobile phase:** Methanol, 1% solution of ammonium acetate, and glacial acetic acid (60:10:0.01)

**Standard solution:** 12.5  $\mu$ g/mL each of USP Buprenorphine Hydrochloride RS and USP Buprenorphine Related Compound A RS in *Mobile phase*

**Sample solution:** 5 mg/mL of buprenorphine hydrochloride in *Mobile phase*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 288 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 3.0 between buprenorphine hydrochloride and buprenorphine related compound A

**Column efficiency:** NLT 6500 theoretical plates

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Allow the *Sample solution* to elute for NLT two times the retention time of buprenorphine hydrochloride. Calculate the percentage of each impurity in the portion of Buprenorphine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response of buprenorphine hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Buprenorphine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Buprenorphine Hydrochloride in the *Sample solution* (mg/mL)

### Acceptance criteria

**Individual impurity:** NMT 0.25%

**Total impurities:** NMT 0.65%

### SPECIFIC TESTS

#### OPTICAL ROTATION, Specific Rotation (781S)

**Sample solution:** 20 mg/mL in methanol

**Acceptance criteria:**  $-92^\circ$  to  $-98^\circ$

#### PH (791)

**Sample:** 10-mg/mL solution

**Acceptance criteria:** 4.0–6.0

#### WATER DETERMINATION, Method I (921): NMT 1.0%

### ADDITIONAL REQUIREMENTS

#### PACKAGING AND STORAGE: Preserve in tight, light-resistant containers.

#### USP REFERENCE STANDARDS (11)

USP Buprenorphine Hydrochloride RS

USP Buprenorphine Related Compound A RS

## Buprenorphine Compounded Buccal Solution, Veterinary

### DEFINITION

Buprenorphine Compounded Buccal Solution, Veterinary, contains NLT 90.0% and NMT 110.0% of the labeled amount of buprenorphine ( $C_{29}H_{41}NO_4$ ).

Prepare Buprenorphine Compounded Buccal Solution, Veterinary 3 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Buprenorphine (as hydrochloride)	30 mg (32.4 mg)
Dextrose	500 mg
Sodium Citrate (anhydrous)	20 mg
Citric Acid Monohydrate	25 mg
Purified Water, a sufficient quantity to make	10 mL



Dissolve the *Dextrose*, *Sodium Citrate Anhydrous*, and *Citric Acid Monohydrate* in 5 mL of *Purified Water* in a suitable calibrated container. Add the *Buprenorphine hydrochloride* powder into the mixture and add sufficient *Purified Water* to bring to final volume, and mix well.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Acetonitrile and 10 mM ammonium acetate (80:20)

**Standard solution:** 0.3 mg/mL of buprenorphine prepared from USP Buprenorphine Hydrochloride RS in methanol

**Sample solution:** Transfer 1 mL of Buccal Solution, Veterinary into a 10-mL volumetric flask, dilute with methanol to volume, and mix well.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 2.1-mm × 5-cm; 5-μm packing L7

**Column temperature:** 40°

**Flow rate:** 0.25 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time for buprenorphine is about 5.8 min.]

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of buprenorphine ( $C_{29}H_{41}NO_4$ ) in the portion of Buccal Solution, Veterinary taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of buprenorphine from the *Sample solution*

$r_s$  = peak response of buprenorphine from the *Standard solution*

$C_s$  = concentration of buprenorphine in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of buprenorphine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

• **pH (791):** 3.5–4.5

**ADDITIONAL REQUIREMENTS**

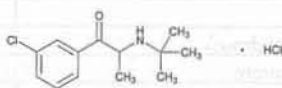
• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8°.

• **LABELING:** Label it to indicate that it is for veterinary use only. Label to indicate that it is for buccal administration, and to state the *Beyond-Use Date*.

• **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored at 2°–8°

• **USP REFERENCE STANDARDS (11)**

USP Buprenorphine Hydrochloride RS

**Bupropion Hydrochloride**

$C_{13}H_{18}ClNO \cdot HCl$

276.20

1-Propanone, 1-(3-chlorophenyl)-2-[(1,1-dimethylethyl)amino]-, hydrochloride, (±)-;  
(±)-2-(*tert*-Butylamino)-3'-chloropropiophenone hydrochloride [31677-93-7].

**DEFINITION**

Bupropion Hydrochloride contains NLT 98.0% and NMT 102.0% of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ), calculated on the anhydrous basis.

**IDENTIFICATION**

• **A. INFRARED ABSORPTION (197K)**

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

• **C. IDENTIFICATION TESTS—GENERAL, Chloride (191)**

**Sample solution:** 1 mg/mL of Bupropion Hydrochloride

**Acceptance criteria:** Meets the requirements for the silver nitrate precipitate test

**ASSAY**• **PROCEDURE**

**Diluent:** Methanol and water (50:50)

**Buffer:** 3.4 g/L of monobasic potassium phosphate in water. Adjust with 1 N sodium hydroxide to a pH of 7.0.

**Mobile phase:** Methanol, tetrahydrofuran, and *Buffer* (39:11:50)

**Standard solution:** 1 mg/mL of USP Bupropion Hydrochloride RS and 2 μg/mL each of USP Bupropion Hydrochloride Related Compound A RS and USP Bupropion Hydrochloride Related Compound B RS in *Diluent*

**Sample solution:** 1 mg/mL of Bupropion Hydrochloride in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 250 nm

**Column:** 3.9-mm × 15-cm; 5-μm packing L7

**Flow rate:** 1.1 mL/min

**Injection volume:** 20 μL

**System suitability**

**Sample:** *Standard solution*

[NOTE—See *Table 3* for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.3 between bupropion hydrochloride related compound A and bupropion; NLT 1.3 between bupropion and bupropion hydrochloride related compound B

**Relative standard deviation:** NMT 2.0% for bupropion

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) in the portion of Bupropion Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Bupropion Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES**

• **LIMIT OF 3-CHLOROBENZOIC ACID**

Protect all analytical solutions from light and use within one day.

**Diluent:** Methanol and 0.001 N hydrochloric acid (20:80)



**Solution A:** Acetonitrile and water (10:90). Add 0.4 mL of trifluoroacetic acid per L of the mixture.

**Solution B:** Acetonitrile and water (95:5). Add 0.3 mL of trifluoroacetic acid per L of the mixture.

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3.4	87	13
10.0	15	85
10.1	0	100
13.0	0	100
13.2	90	10
19.0	90	10

**System suitability stock solution:** 0.02 mg/mL of USP Bupropion Hydrochloride Related Compound C RS, 0.02 mg/mL of USP Bupropion Hydrochloride Related Compound F RS, and 0.012 mg/mL of USP 3-Chlorobenzoic Acid RS in methanol

**System suitability solution:** 0.002 mg/mL of bupropion hydrochloride related compound C, 0.002 mg/mL of bupropion hydrochloride related compound F, and 0.0012 mg/mL of 3-chlorobenzoic acid from *System suitability stock solution* in *Diluent*

**Standard stock solution:** 0.06 mg/mL of USP 3-Chlorobenzoic Acid RS in methanol

**Standard solution:** 1.2 µg/mL of USP 3-Chlorobenzoic Acid RS from *Standard stock solution* in *Diluent*

**Sample solution:** 600 µg/mL of Bupropion Hydrochloride in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 226 nm

**Column:** 4.6-mm × 10-cm; 3.5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 5 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.3 between bupropion hydrochloride related compound F and bupropion hydrochloride related compound C, *System suitability solution*; NLT 1.5 between bupropion hydrochloride related compound C and 3-chlorobenzoic acid, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 3-chlorobenzoic acid in the portion of Bupropion Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of 3-chlorobenzoic acid from the *Sample solution*

$r_S$  = peak response of 3-chlorobenzoic acid from the *Standard solution*

$C_S$  = concentration of USP 3-Chlorobenzoic Acid RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Bupropion Hydrochloride in the *Sample solution* (µg/mL)

**Acceptance criteria:** See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Bupropion	1.0	—
Bupropion hydrochloride related compound F <sup>a</sup>	1.71	—
Bupropion hydrochloride related compound C <sup>a</sup>	1.75	—
3-Chlorobenzoic acid	1.80	0.2

<sup>a</sup> Included for system suitability purposes only.

#### • ORGANIC IMPURITIES

**Diluent, Buffer, Mobile phase, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

#### System suitability

**Sample:** *Standard solution*

[NOTE—See Table 3 for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.3 between bupropion hydrochloride related compound A and bupropion; NLT 1.3 between bupropion and bupropion hydrochloride related compound B

**Relative standard deviation:** NMT 2.0% for bupropion; NMT 5.0% for bupropion hydrochloride related compound B

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Bupropion Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response for bupropion from the *Standard solution*

$C_S$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Bupropion Hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor for each impurity relative to bupropion (see Table 3)

**Acceptance criteria:** See Table 3.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Deschloro bupropion <sup>a</sup>	0.38	1.5	0.5
Bupropion dione derivative <sup>b</sup>	0.58	1.0	0.2
o-Bupropion <sup>c</sup>	0.71	0.45	0.1
Chloropropiophenone <sup>d</sup>	0.78	1.2	0.1

<sup>a</sup> 2-(*tert*-Butylamino)-1-phenylpropan-1-one; also known as 2-(*tert*-butylamino)propiofenone.

<sup>b</sup> 1-(3-Chlorophenyl)propane-1,2-dione; also known as 1-(3-chlorophenyl)-1,2-propanedione.

<sup>c</sup> 2-(*tert*-Butylamino)-1-(2-chlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-2'-chloropropiophenone.

<sup>d</sup> 1-(3-Chlorophenyl)propan-1-one; also known as 3'-chloropropiophenone.

<sup>e</sup> 2-Bromo-1-(3-chlorophenyl)propan-1-one; also known as 2-bromo-3'-chloropropiophenone.

<sup>f</sup> 2-(*tert*-Butylamino)-1-(3,4-dichlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-3',4'-dichloropropiophenone.

<sup>g</sup> 2-(*tert*-Butylamino)-1-(3,5-dichlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-3',5'-dichloropropiophenone.

<sup>h</sup> Sum of all impurities found in the tests for *Limit of 3-Chlorobenzoic Acid* and *Organic Impurities*.



Table 3 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bupropion hydrochloride related compound A	0.92	1.4	0.2
Bupropion	1.0	—	—
Bupropion hydrochloride related compound B	1.14	0.81	0.2
Bromochloropropiophenone <sup>c</sup>	1.63	0.88	0.1
4-Chlorobupropion <sup>f</sup>	2.30	1.1	0.2
5-Chlorobupropion <sup>g</sup>	2.74	0.69	0.2
Any individual impurity	—	1.0	0.1
Total impurities <sup>h</sup>	—	—	1.0

<sup>a</sup> 2-(*tert*-Butylamino)-1-phenylpropan-1-one; also known as 2-(*tert*-butylamino)propiophenone.

<sup>b</sup> 1-(3-Chlorophenyl)propane-1,2-dione; also known as 1-(3-chlorophenyl)-1,2-propanedione.

<sup>c</sup> 2-(*tert*-Butylamino)-1-(2-chlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-2'-chloropropiophenone.

<sup>d</sup> 1-(3-Chlorophenyl)propan-1-one; also known as 3'-chloropropiophenone.

<sup>e</sup> 2-Bromo-1-(3-chlorophenyl)propan-1-one; also known as 2-bromo-3'-chloropropiophenone.

<sup>f</sup> 2-(*tert*-Butylamino)-1-(3,4-dichlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-3',4'-dichloropropiophenone.

<sup>g</sup> 2-(*tert*-Butylamino)-1-(3,5-dichlorophenyl)propan-1-one; also known as 2-(*tert*-butylamino)-3',5'-dichloropropiophenone.

<sup>h</sup> Sum of all impurities found in the tests for Limit of 3-Chlorobenzoic Acid and Organic Impurities.

## SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 0.5%

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at room temperature.
- **USP REFERENCE STANDARDS (11)**
  - USP Bupropion Hydrochloride RS
  - USP Bupropion Hydrochloride Related Compound A RS
  - 2-(*tert*-Butylamino)-4'-chloropropiophenone hydrochloride.  
 $C_{13}H_{18}ClNO \cdot HCl$  276.20
  - USP Bupropion Hydrochloride Related Compound B RS
  - 2-(*tert*-Butylamino)-3'-bromopropiophenone hydrochloride.  
 $C_{13}H_{18}BrNO \cdot HCl$  320.66
  - USP Bupropion Hydrochloride Related Compound C RS
  - 1-(3-Chlorophenyl)-2-hydroxypropan-1-one.  
 $C_9H_9O_2Cl$  184.62
  - USP Bupropion Hydrochloride Related Compound F RS
  - 1-(3-Chlorophenyl)-1-hydroxypropan-2-one.  
 $C_9H_9O_2Cl$  184.62
  - USP 3-Chlorobenzoic Acid RS
  - 3-Chlorobenzoic acid.  
 $C_7H_5ClO_2$  156.57

## Bupropion Hydrochloride Tablets

### DEFINITION

Bupropion Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ).

## IDENTIFICATION

### A. INFRARED ABSORPTION (197K)

**Sample:** Crush 1 Tablet using a mortar and pestle. Prepare an approximate 1% (w/w) dispersion of the sample in potassium bromide.

**Acceptance criteria:** The *Sample* shows strong bands at about 1690, 1560, and 1240  $cm^{-1}$  and a weaker band at about 740  $cm^{-1}$ , similar to the reference preparation.

- **B.** The retention time of the major peak of the *Sample* solution corresponds to that of the *Standard* solution, as obtained in the Assay.

## ASSAY

### PROCEDURE

**Buffer:** 6.8 g/L of monobasic potassium phosphate and 1.164 g/L of sodium hydroxide in water

**Mobile phase:** Methanol and *Buffer* (65:35)

**Diluent:** Methanol and water (65:35)

**Standard solution:** 0.6 mg/mL of USP Bupropion Hydrochloride RS in *Diluent*

**Sample stock solution:** Nominally 3.0 mg/mL of bupropion hydrochloride in *Diluent* prepared as follows. Transfer an appropriate number of Tablets to a suitable volumetric flask. Add 50% of the flask volume of *Diluent*, and shake by mechanical means until the Tablets have disintegrated (30–60 min). Sonicate for 5 min, dilute with *Diluent* to volume, and mix. Allow to stand for at least 30 min. Use the supernatant.

**Sample solution:** Nominally 0.6 mg/mL of bupropion hydrochloride from the *Sample stock solution* in *Diluent*

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 224 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m base-deactivated packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 2.0%

**Analysis**

Calculate the percentage of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bupropion hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

### DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Standard solution:** USP Bupropion Hydrochloride RS at a known concentration in 0.1 N hydrochloric acid

**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with 0.1 N hydrochloric acid, if necessary.



**Instrumental conditions**

Mode: UV

Analytical wavelength: 252 nm

**Analysis****Samples:** *Standard solution* and *Sample solution***Tolerances:** NLT 80% (Q) of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
USP Bupropion Hydrochloride RS

## Bupropion Hydrochloride Extended-Release Tablets

**DEFINITION**

Bupropion Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**

**Sample:** Crush 1 Tablet using a mortar and pestle. Prepare an approximate 1% (w/w) dispersion of the sample in potassium bromide.

**Acceptance criteria:** The *Sample* shows strong bands at about 1690, 1560, and 1240  $cm^{-1}$  and a weaker band at about 740  $cm^{-1}$ , similar to the reference preparation.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

- **PROCEDURE**

**Diluent 1:** Methanol and 0.001 N hydrochloric acid (20:80)

**Solution A:** Acetonitrile, trifluoroacetic acid, and water (10:0.04:90)

**Solution B:** Acetonitrile, trifluoroacetic acid, and water (95:0.03:5)

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
3.4	87	13
10.0	15	85
10.1	0	100
13.0	0	100
13.2	90	10
19.0	90	10

**System suitability stock solution:** 0.02 mg/mL of USP Bupropion Hydrochloride Related Compound C RS and 0.2 mg/mL of USP Bupropion Hydrochloride Related Compound F RS in methanol

**System suitability solution:** 0.002 mg/mL of bupropion hydrochloride related compound C and 0.02 mg/mL of bupropion hydrochloride related compound F from the *System suitability stock solution* in *Diluent 1*

**Standard solution:** 0.6 mg/mL of USP Bupropion Hydrochloride RS in *Diluent 1*

**Sample stock solution A:** Transfer a number of Tablets, intact or crushed, to a suitable homogenizer vessel containing sufficient methanol to obtain a concentration of

3.0 mg/mL of bupropion hydrochloride. Immediately homogenize the sample for 30 s at 20,000 rpm. Allow extraction for 3 min, and follow by two additional 10-s pulses, each at 20,000 rpm, pausing 3 min between these pulses to ensure complete extraction. Pass a portion of the solution through a nylon filter of 0.45- $\mu$ m pore size, discarding the first 2–4 mL of the filtrate.

**Sample solution A:** Nominally 0.6 mg/mL of bupropion hydrochloride from *Sample stock solution A* in 0.001 N hydrochloric acid

Alternatively, the *Sample solution* can be prepared as follows.

**Buffer:** Dissolve 100 g of anhydrous disodium hydrogen phosphate in 1 L of water. Add 50 mL of phosphoric acid, stir or sonicate until dissolved, and mix. Adjust with phosphoric acid to a pH of 3.0.

**Diluent 2:** Methanol and *Buffer* (20:80)

**Sample stock solution B:** Weigh and grind NLT 20

Tablets to prepare a solution having a nominal concentration of 3 mg/mL. Initially add *Diluent 2* (75% of the volume of the flask), stir for 30 min, and sonicate for 15 min. Dilute with *Diluent 2* to volume. Centrifuge a portion of the resulting solution, and use the supernatant.

**Sample solution B:** Nominally 0.6 mg/mL of bupropion hydrochloride from *Sample stock solution B* in *Diluent 2*

**Chromatographic system**

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 226 nm

**Column:** 4.6-mm  $\times$  10-cm; 3.5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 5  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 16 for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.3 between bupropion hydrochloride related compound F and bupropion hydrochloride related compound C, *System suitability solution*

**Tailing factor:** NMT 1.9, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution A* or *Sample solution B*

Calculate the percentage of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of bupropion hydrochloride from *Sample solution A* or *Sample solution B*

$r_S$  = peak response of bupropion hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of bupropion hydrochloride in *Sample solution A* or *Sample solution B* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **DISSOLUTION (711)**

For products labeled for dosing every 12 h

**Test 1**

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Times:** 1, 4, and 8 h

**Standard solution:** (L/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where L is the label claim, in mg/Tablet. Dilute with *Medium*, if necessary.



**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 298 nm

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved.

**Tolerances:** See Table 2.

**Table 2**

Time (h)	Amount Dissolved
1	25%–45%
4	60%–85%
8	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.1 N hydrochloric acid, pH 1.5 (prepared by transferring 50 mL of concentrated hydrochloric acid to 6000 mL of water, adding 18 g of sodium hydroxide, mixing, and adjusting with either diluted sodium hydroxide or hydrochloric acid to a pH of 1.5); 900 mL, deaerated

**Apparatus 1:** 50 rpm

**Times:** 1, 2, 4, and 6 h

**Buffer:** 3.45 g of monobasic sodium phosphate monohydrate in 996 mL of water. Add 4.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.80.

**Mobile phase:** Methanol and *Buffer* (35:65)

**Standard solution:** ( $L/900$ ) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where  $L$  is the label claim, in mg/Tablet

**Sample solution:** Use portions of the solution under test, and pass through a nylon filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 298 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 2000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved.

**Tolerances:** See Table 3.

**Table 3**

Time (h)	Amount Dissolved
1	25%–50%
2	40%–65%

**Table 3 (Continued)**

Time (h)	Amount Dissolved
4	65%–90%
6	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm. Use wire coil sinkers, if necessary.

**Times:** 1, 2, 4, and 6 h

**Standard solution:** ( $L/900$ ) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where  $L$  is the label claim, in mg/Tablet. Dilute with *Medium*, if necessary.

**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 250 nm

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved.

**Tolerances:** See Table 4.

**Table 4**

Time (h)	Amount Dissolved (for Tablets that contain 200 mg of bupropion hydrochloride)	Amount Dissolved (for Tablets that contain all other strengths of bupropion hydrochloride)
1	30%–50%	30%–55%
2	45%–65%	50%–75%
4	65%–85%	70%–90%
6	NLT 78%	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**Test 5:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Times:** 1, 3, and 6 h

**Standard solution:** ( $L/900$ ) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where  $L$  is the label claim, in mg/Tablet. Dilute with *Medium*, if necessary.

**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 298 nm

**Cell:** 0.5 cm

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved.



Tolerances: See Table 5.

Table 5

Time (h)	Amount Dissolved
1	35%–55%
3	65%–85%
6	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to Acceptance Table 2 in (711).

**Test 7:** If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 7.

**Medium:** 0.1 N hydrochloric acid, pH 1.5 (prepared by transferring 50 mL of concentrated hydrochloric acid to 6000 mL of water, adding 18 g of sodium hydroxide, mixing, and adjusting with either diluted sodium hydroxide or hydrochloric acid to a pH of 1.5); 900 mL, deaerated

**Apparatus 1:** 50 rpm

**Times:** 1, 2, 4, and 6 h

**Buffer:** 3.45 g of monobasic sodium phosphate monohydrate in 996 mL of water. Add 4.0 mL of triethylamine, and adjust with phosphoric acid to a pH of 2.80.

**Mobile phase:** Methanol and Buffer (45:55)

**Standard solution:** ( $L/900$ ) mg/mL of USP Bupropion Hydrochloride RS in Medium, where  $L$  is the label claim, in mg/Tablet

**Sample solution:** Use portions of the solution under test, and pass through a nylon filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 298 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** Standard solution

**Suitability requirements**

**Column efficiency:** NLT 2000 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** Standard solution and Sample solution

Determine the percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved.

Tolerances: See Table 6.

Table 6

Time (h)	Amount Dissolved
1	25%–50%
2	45%–70%
4	NLT 70%
6	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to Acceptance Table 2 in (711).

**Test 9:** If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 9.

**Medium:** 0.1 N hydrochloric acid, pH 1.5 (prepared by transferring 50 mL of concentrated hydrochloric acid to 6000 mL of water, adding 18 g of sodium hydroxide, mixing, and adjusting with either diluted so-

dium hydroxide or hydrochloric acid to a pH of 1.5); 900 mL

**Apparatus 1:** 50 rpm

**Times:** 1, 2, 4, and 8 h

**Standard solution:** ( $L/1000$ ) mg/mL of USP Bupropion Hydrochloride RS in Medium, where  $L$  is the label claim, in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Instrumental conditions**

(See Ultraviolet-Visible Spectroscopy (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 298 nm

**Blank:** Medium

**Analysis**

**Samples:** Standard solution and Sample solution

Determine the percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved.

Tolerances: See Table 7.

Table 7

Time (h)	Amount Dissolved
1	20%–45%
2	35%–55%
4	55%–85%
8	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to Acceptance Table 2 in (711).

**Test 10:** If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 10.

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Times:** 1, 2, 4, and 8 h

**Standard solution:** ( $L/900$ ) mg/mL of USP Bupropion Hydrochloride RS in Medium, where  $L$  is the label claim, in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Instrumental conditions**

(See Ultraviolet-Visible Spectroscopy (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 298 nm

**Cell:** 0.5 cm

**Blank:** Medium

**System suitability**

**Sample:** Standard solution

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_i = (A_i/A_s) \times C_s \times V \times (1/L) \times 100$$

$A_i$  = absorbance of bupropion hydrochloride from the Sample solution at time point  $i$

$A_s$  = absorbance of bupropion hydrochloride from the Standard solution

$C_s$  = concentration of USP Bupropion Hydrochloride RS in the Standard solution (mg/mL)

$V$  = volume of Medium, 900 mL

$L$  = label claim (mg/Tablet)

Tolerances: See Table 8.



Table 8

Time Point (h)	Time (h)	Amount Dissolved
1	1	20%–40%
2	2	35%–60%
3	4	55%–85%
4	8	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**For products labeled for dosing every 24 h**

**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Medium:** 0.1 N hydrochloric acid; 900 mL, deaerated

**Apparatus 1:** 75 rpm

**Times:** 2, 4, 8, and 16 h

**Standard solution:** ( $L/900$ ) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where  $L$  is the label claim, in mg/Tablet. Dilute with *Medium*, if necessary.

**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 252 nm

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved.

**Tolerances:** See *Table 9*.

Table 9

Time (h)	Amount Dissolved
2	NMT 20%
4	20%–45%
8	65%–90%
16	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**Test 6:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

**Medium:** 0.1 N hydrochloric acid; 900 mL, deaerated

**Apparatus 1:** 75 rpm

**Times:** 1, 2, 4, 8, and 12 h

**Standard solution:** ( $L/900$ ) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where  $L$  is the label claim, in mg/Tablet. Dilute with *Medium*, if necessary.

**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with *Medium*, if necessary.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 298 nm

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved.

**Tolerances:** See *Table 10*.

Table 10

Time (h)	Amount Dissolved
1	15%–35%
2	25%–50%
4	40%–65%
8	65%–90%
12	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**Test 8:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 8*.

**Acid stage medium:** 0.1 N hydrochloric acid; 900 mL

**Buffer stage medium:** pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

**Apparatus 1:** 75 rpm

**Times:** 2 h in *Acid stage medium*; 3, 8, and 16 h in *Buffer stage medium*. The time in the *Buffer stage medium* includes the time in the *Acid stage medium*.

**Standard solution:** ( $L/900$ ) mg/mL of USP Bupropion Hydrochloride RS in *Acid stage medium*, where  $L$  is the label claim, in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 298 nm

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved.

**Tolerances:** See *Table 11*.

Table 11

Time (h)	Amount Dissolved
2	NMT 10%
3	10%–30%
8	60%–90%
16	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**Test 11:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 11*.

**Acid stage medium:** 0.1 N hydrochloric acid; 750 mL

**Buffer stage medium:** pH 6.8 phosphate buffer (Add 250 mL of 0.2 M tribasic sodium phosphate to the *Acid stage medium*, adjust with 2 N hydrochloric acid or 2 N sodium hydroxide to a pH of 6.8, if necessary.); 1000 mL

**Apparatus 2:** 50 rpm

**Times:** 2 h in *Acid stage medium*; 3, 8, and 16 h in *Buffer stage medium*. The time in the *Buffer stage medium* includes the time in the *Acid stage medium*.

**Acid stage standard solution:** 0.06 mg/mL of USP Bupropion Hydrochloride RS in *Acid stage medium*. Sonication may be used to aid in dissolution.

**Buffer stage standard solution:** 0.15 mg/mL of USP Bupropion Hydrochloride RS in *Buffer stage medium*. Sonication may be used to aid in dissolution.



**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 298 nm

**Cell:** 0.5 cm

**Blank:** Acid stage medium or Buffer stage medium

**Analysis**

**Samples:** Acid stage standard solution, Buffer stage standard solution, and Sample solution

Calculate the concentration ( $C_i$ ) of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) in the sample withdrawn from the vessel at time point  $i$ :

$$\text{Result}_i = (A_i/A_s) \times C_s$$

$A_i$  = absorbance of bupropion hydrochloride from the Sample solution at time point  $i$

$A_s$  = absorbance of bupropion hydrochloride from the Acid stage standard solution or Buffer stage standard solution

$C_s$  = concentration of USP Bupropion Hydrochloride RS in the Acid stage standard solution or Buffer stage standard solution (mg/mL)

Calculate the percentage of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_1 \times V_A \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V_B - V_s)] + (C_1 \times V_s)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times (V_B - (2 \times V_s))] + [(C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times (V_B - (3 \times V_s))] + [(C_3 + C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$C_i$  = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point  $i$  (mg/mL)

$V_A$  = volume of Acid stage medium, 750 mL

$L$  = label claim (mg/Tablet)

$V_B$  = volume of Buffer stage medium, 1000 mL

$V_s$  = volume of Sample solution withdrawn from the Acid stage medium or Buffer stage medium (mL)

**Tolerances:** See Table 12.

**Table 12**

Time Point (i)	Time (h)	Amount Dissolved
1	2	NMT 10%
2	3	10%–30%
3	8	55%–85%
4	16	NLT 75%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to Acceptance Table 2 in (711).

**Test 12:** If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 12.

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 75 rpm

**Times:** 2, 4, 8, and 12 h

**Standard solution:** (L/900) mg/mL of USP Bupropion Hydrochloride RS in Medium, where L is the label claim, in mg/Tablet

**Sample solution:** Withdraw at least 10 mL of the solution under test and pass through a suitable filter.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 252 nm

**Cell**

For Tablets labeled to contain 150 mg: 0.1 cm

For Tablets labeled to contain 300 mg: 0.05 cm

**Blank:** Medium

**System suitability**

**Sample:** Standard solution

**Suitability requirements**

Relative standard deviation: NMT 3.0%

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the concentration ( $C_i$ ) of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) in the sample withdrawn from the vessel at time point  $i$ :

$$\text{Result}_i = (A_i/A_s) \times C_s$$

$A_i$  = absorbance of bupropion hydrochloride from the Sample solution at time point  $i$

$A_s$  = absorbance of bupropion hydrochloride from the Standard solution

$C_s$  = concentration of USP Bupropion Hydrochloride RS in the Standard solution (mg/mL)

Calculate the percentage of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_s)] + (C_1 \times V_s)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times (V - (2 \times V_s))] + [(C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times (V - (3 \times V_s))] + [(C_3 + C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$C_i$  = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point  $i$  (mg/mL)

$V$  = volume of Medium, 900 mL

$L$  = label claim (mg/Tablet)

$V_s$  = volume of Sample solution withdrawn from the Medium (mL)

**Tolerances:** See Table 13.

**Table 13**

Time Point (i)	Time (h)	Amount Dissolved
1	2	NMT 25%
2	4	25%–50%
3	8	60%–85%
4	12	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to Acceptance Table 2 in (711).

**Test 13:** If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 13.



**Medium:** 0.1 N hydrochloric acid; 900 mL, deaerated

**Apparatus 1:** 75 rpm

**Times:** 2, 4, 8, and 12 h

**Standard solution:** (L/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where L is the label claim, in mg/Tablet

**Sample solution:** Withdraw at least 10 mL of the solution under test and centrifuge. Use the supernatant.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV-Vis

**Analytical wavelength:** 252 nm

**Cell:** 0.1 cm

**Blank:** *Medium*

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration ( $C_i$ ) of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) in the sample withdrawn from the vessel at time point  $i$ :

$$Result_i = (A_i/A_5) \times C_5$$

$A_i$  = absorbance of bupropion hydrochloride from the *Sample solution* at time point  $i$

$A_5$  = absorbance of bupropion hydrochloride from the *Standard solution*

$C_5$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$Result_1 = C_i \times V \times (1/L) \times 100$$

$$Result_2 = [(C_2 \times (V - V_3)) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$Result_3 = [(C_3 \times (V - (2 \times V_3))] + [(C_2 + C_1) \times V_3] \times (1/L) \times 100$$

$$Result_4 = [(C_4 \times (V - (3 \times V_3))] + [(C_3 + C_2 + C_1) \times V_3] \times (1/L) \times 100$$

$C_i$  = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point  $i$  (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

$V_3$  = volume of *Sample solution* withdrawn from the *Medium* (mL)

**Tolerances:** See Table 14.

**Table 14**

Time Point (i)	Time (h)	Amount Dissolved (150 mg/Tablet)	Amount Dissolved (300 mg/Tablet)
1	2	NMT 25%	NMT 25%
2	4	30%–55%	25%–45%
3	8	65%–90%	60%–80%
4	12	NLT 80%	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

**Test 14:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 14*.

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 75 rpm

**Times:** 2, 4, 8, and 16 h

**Standard solution:** (L/900) mg/mL of USP Bupropion Hydrochloride RS in *Medium*, where L is the label claim, in mg/Tablet. If necessary, dilute the solution with *Medium*.

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Replace the portion removed with the same volume of *Medium*. If necessary, dilute the filtrate with *Medium*.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV

**Analytical wavelength:** 252 nm

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration ( $C_i$ ) of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) in the sample withdrawn from the vessel at time point  $i$ :

$$Result_i = (A_i/A_5) \times C_5 \times D$$

$A_i$  = absorbance from the *Sample solution* at time point  $i$

$A_5$  = absorbance from the *Standard solution*

$C_5$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

$D$  = dilution factor for the *Sample solution*, if needed

Calculate the percentage of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$Result_1 = C_i \times V \times (1/L) \times 100$$

$$Result_2 = [(C_2 \times V) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$Result_3 = [(C_3 \times V) + [(C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

$$Result_4 = [(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

$C_i$  = concentration of bupropion hydrochloride in the portion of the sample withdrawn at time point  $i$  (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

$V_3$  = volume of *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

**Tolerances:** See Table 15.

**Table 15**

Time Point (i)	Time (h)	Amount Dissolved
1	2	NMT 20%
2	4	20%–45%
3	8	55%–85%
4	16	NLT 80%

The percentages of the labeled amount of bupropion hydrochloride ( $C_{13}H_{18}ClNO \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements



**IMPURITIES****• ORGANIC IMPURITIES**

**Diluent 1, Solution A, Solution B, Mobile phase, and Sample solution A or Sample solution B:** Proceed as directed in the Assay.

**System suitability stock solution A:** 0.02 mg/mL of USP Bupropion Hydrochloride Related Compound C RS, 0.02 mg/mL of USP Bupropion Hydrochloride Related Compound F RS, and 0.012 mg/mL of USP 3-Chlorobenzoic Acid RS in methanol

**System suitability solution A:** 0.002 mg/mL of bupropion hydrochloride related compound C, 0.002 mg/mL of bupropion hydrochloride related compound F, and 0.0012 mg/mL of 3-chlorobenzoic acid from *System suitability stock solution A* in *Diluent 1*

**System suitability stock solution B:** 0.012 mg/mL of USP 3-Chlorobenzoic Acid RS in methanol

**System suitability solution B:** 0.0012 mg/mL of 3-chlorobenzoic acid from *System suitability stock solution B* in *Diluent 1*

**Standard solution:** 0.0012 mg/mL of USP Bupropion Hydrochloride RS in *Diluent 1*

**Chromatographic system:** Proceed as directed in the Assay except use a *Detector* as follows:

**Detector:** UV 226 nm, adjusted  $\pm 2$  nm so that the relative response factor requirement is met. [NOTE—The peak responses of the compounds of interest are very sensitive to changes in the detection wavelength.]

**System suitability**

**Samples:** *System suitability solution A*, *System suitability solution B*, and *Standard solution*

[NOTE—See *Table 16* for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.3 between bupropion hydrochloride related compound F and bupropion hydrochloride related compound C, *System suitability solution A*; NLT 1.3 between bupropion hydrochloride C and 3-chlorobenzoic acid, *System suitability solution A*

**Relative standard deviation:** NMT 10%, *Standard solution*

**Relative response factor:** 3.8–4.5 for the peak response of 3-chlorobenzoic acid in *System suitability solution B* divided by the peak response from bupropion in the *Standard solution*

**Analysis**

**Samples:** *System suitability solution B*, *Standard solution*, and *Sample solution A* or *Sample solution B*

Calculate the percentage of 3-chlorobenzoic acid in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of 3-chlorobenzoic acid from *Sample solution A* or *Sample solution B*

$r_s$  = peak response of 3-chlorobenzoic acid from *System suitability solution B*

$C_s$  = concentration of USP 3-Chlorobenzoic Acid RS in *System suitability solution B* (mg/mL)

$C_u$  = nominal concentration of bupropion hydrochloride in *Sample solution A* or *Sample solution B* (mg/mL)

Calculate the percentage of each other degradation product in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of each other degradation product from *Sample solution A* or *Sample solution B*

$r_s$  = peak response of bupropion hydrochloride from the *Standard solution*

$C_s$  = concentration of USP Bupropion Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of bupropion hydrochloride in *Sample solution A* or *Sample solution B* (mg/mL)

$F$  = relative response factor for each other degradation product (see *Table 16*)

**Acceptance criteria:** See *Table 16*.

**Table 16**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)	
			100 mg or less	150 mg or greater
Bupropion amine <sup>a</sup>	0.38	1.2	0.3	0.3
S,S,S-Thiomorpholine derivative <sup>b</sup>	0.56	1.1	1.0	1.5
S,R,R-Thiomorpholine derivative <sup>c</sup>	0.78	1.1	0.5	0.4
Bupropion	1.0	—	—	—
Bupropion related compound F	1.71	1.8	1.2	2.3
Bupropion related compound C	1.75	1.7	0.3	0.3
3-Chlorobenzoic acid	1.80	—	0.3	0.3
Bupropion dione derivative <sup>d</sup>	2.25	1.00	0.4	0.4
Any unspecified degradation product	—	1.00	0.2	0.2
Total impurities	—	—	3.2	3.3

<sup>a</sup> 2-Amino-1-(3-chlorophenyl)-1-propanone.

<sup>b</sup> (3S,5S,6S)-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid.

<sup>c</sup> (3S,5R,6R)-6-(3-Chlorophenyl)-6-hydroxy-5-methyl-3-thiomorpholine carboxylic acid.

<sup>d</sup> 1-(3-Chlorophenyl)propane-1,2-dione.

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature. Protect from light.

**• LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

**• USP REFERENCE STANDARDS (11)**

USP Bupropion Hydrochloride RS

USP Bupropion Hydrochloride Related Compound C RS  
1-(3-Chlorophenyl)-2-hydroxypropan-1-one.

$C_9H_9O_2Cl$  184.62

USP Bupropion Hydrochloride Related Compound F RS  
1-(3-Chlorophenyl)-1-hydroxypropan-2-one.

$C_9H_9O_2Cl$  184.62

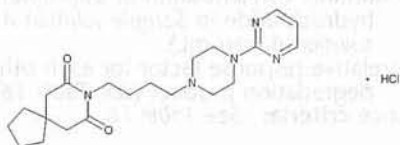
USP 3-Chlorobenzoic Acid RS

3-Chlorobenzoic acid.

$C_7H_5ClO_2$  156.57



## Buspirone Hydrochloride



$C_{21}H_{31}N_5O_2 \cdot HCl$  421.96  
8-Azaspiro[4,5]decane-7,9-dione, 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-, monohydrochloride;  
N-[4-[4-(2-Pyrimidinyl)-1-piperazinyl]butyl]-1,1-cyclopentanediacetamide monohydrochloride [33386-08-2].

### DEFINITION

Buspirone Hydrochloride contains NLT 97.5% and NMT 102.5% of buspirone hydrochloride ( $C_{21}H_{31}N_5O_2 \cdot HCl$ ), calculated on the as-is basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The relative retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride** (191)  
*Sample solution:* 10 mg/mL in water  
*Acceptance criteria:* Meets the requirements

### ASSAY

#### PROCEDURE

**Buffer A:** 6.8 g/L of monobasic potassium phosphate and 0.93 g/L of sodium 1-hexanesulfonate monohydrate. Adjust with phosphoric acid to a pH of 3.4.

**Buffer B:** 3.4 g/L of monobasic potassium phosphate and 3.52 g/L of sodium 1-hexanesulfonate monohydrate. Adjust with phosphoric acid to a pH of 2.2.

**Solution A:** Acetonitrile and *Buffer A* (5:95)

**Solution B:** Acetonitrile and *Buffer B* (75:25)

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
6	90	10
34	42	58
45	42	58
55	0	100
56	100	0
60	100	0
61	90	10

**Diluent:** *Solution A*

**Impurities stock solution:** 0.25 mg/mL each of USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in acetonitrile

**System suitability solution:** 1.0 mg/mL of USP Buspirone Hydrochloride RS and 0.001 mg/mL each of USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in *Diluent*, from *Impurities stock solution*

**Standard solution:** 0.1 mg/mL of USP Buspirone Hydrochloride RS in *Diluent*

**Sample solution:** 0.1 mg/mL of Buspirone Hydrochloride in *Diluent*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 20 μL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 2.0 between buspirone and buspirone related compound G peaks, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 0.92%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of buspirone hydrochloride ( $C_{21}H_{31}N_5O_2 \cdot HCl$ ) in the portion of Buspirone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Buspirone Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.5%–102.5% on the as-is basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.5%

### Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1-Jan-2018)

### ORGANIC IMPURITIES

**Buffer A, Buffer B, Solution A, Solution B, Mobile phase, Diluent, Impurities stock solution, and System suitability solution:** Proceed as directed in the *Assay*.

**Standard solution:** 0.001 mg/mL each of USP Buspirone Hydrochloride RS, USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in *Diluent*

**Sample solution:** 1.0 mg/mL of Buspirone Hydrochloride in *Diluent*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 and 240 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 20 μL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* and *Table 3* for relative retention times.]

### Suitability requirements

**Resolution at 240 nm:** NLT 2.0 between buspirone and buspirone related compound G peaks, *System suitability solution*



**Resolution at 210 nm:** NLT 4.0 between buspirone related compound L and buspirone related compound N peaks, *System suitability solution*  
**Relative standard deviation:** NMT 2.0% for each peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
**For impurities detected at UV 240 nm**

Calculate the percentage of buspirone related compound A or buspirone related compound G in the portion of Buspirone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of buspirone related compound A or buspirone related compound G from the *Sample solution*

$r_S$  = peak response of buspirone related compound A or buspirone related compound G from the *Standard solution*

$C_S$  = concentration of USP Buspirone Related Compound A RS or USP Buspirone Related Compound G RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Buspirone Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of specified impurities and any other individual impurity in the portion of Buspirone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of specified impurities and any other individual impurity from the *Sample solution*

$r_S$  = peak response of buspirone from the *Standard solution*

$C_S$  = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Buspirone Hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see Table 2)

#### Acceptance criteria

**For impurities detected at UV 240 nm:** See Table 2.  
 Disregard any peak below 0.05%.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Buspirone related compound A <sup>a</sup>	0.2	—	0.10
Spiroammonium salt <sup>b</sup>	0.3	1.0	0.10
Bispyrimidinylpiperazinyl butane <sup>c</sup>	0.6	1.0	0.10
Bispyrimidinylpiperazinylbutyl ether <sup>d</sup>	0.7	1.0	0.10
Buspirone open ring <sup>e</sup>	0.8	1.0	0.3

<sup>a</sup> 2-(Piperazin-1-yl)pyrimidine.

<sup>b</sup> 8-(Pyrimidin-2-yl)-8-aza-5-azoniaspiro[4.5]decane.

<sup>c</sup> 1,4-Bis[4-(pyrimidin-2-yl)piperazin-1-yl]butane.

<sup>d</sup> Bis[4-[1-(pyrimidin-2-yl)piperazine-4-yl]butane-1-yl] ether.

<sup>e</sup> 2-[1-[2-Oxo-2-((4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl)amino)ethyl]cyclopentyl]acetic acid.

<sup>f</sup> 4-[4-(Pyrimidin-2-yl)piperazin-1-yl]butyl 2-[1-[2-oxo-2-((4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl)amino)ethyl]cyclopentyl]acetate.

<sup>g</sup> 1,4-Di(piperidin-2-yl)piperazine.

<sup>h</sup> Bis[4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl] 2,2'-(cyclopentane-1,1-diyl)diacetate.

<sup>i</sup> 8-[4-[4-(5-Chloropyrimidin-2-yl)piperazin-1-yl]butyl]-8-azaspiro[4.5]decane-7,9-dione.

<sup>j</sup> 4-(7,9-Dioxo-8-azaspiro[4.5]decan-8-yl)butyl 2-[1-[2-oxo-2-((4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl)amino)ethyl]cyclopentyl]acetate.

**Table 2 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Buspirone open ring dimer <sup>f</sup>	0.9	1.0	0.10
Buspirone	1.0	—	—
Buspirone related compound G <sup>g</sup>	1.05	—	0.10
Buspirone diester dimer <sup>h</sup>	1.1	1.0	0.10
Chlorobuspirone <sup>i</sup>	1.2	1.0	0.10
Buspirone open ring spirodimer <sup>j</sup>	1.5	0.5	0.2
Any other individual impurity	—	1.0	0.10
Total impurities	—	—	0.4

<sup>a</sup> 2-(Piperazin-1-yl)pyrimidine.

<sup>b</sup> 8-(Pyrimidin-2-yl)-8-aza-5-azoniaspiro[4.5]decane.

<sup>c</sup> 1,4-Bis[4-(pyrimidin-2-yl)piperazin-1-yl]butane.

<sup>d</sup> Bis[4-[1-(pyrimidin-2-yl)piperazine-4-yl]butane-1-yl] ether.

<sup>e</sup> 2-[1-[2-Oxo-2-((4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl)amino)ethyl]cyclopentyl]acetic acid.

<sup>f</sup> 4-[4-(Pyrimidin-2-yl)piperazin-1-yl]butyl 2-[1-[2-oxo-2-((4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl)amino)ethyl]cyclopentyl]acetate.

<sup>g</sup> 1,4-Di(piperidin-2-yl)piperazine.

<sup>h</sup> Bis[4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl] 2,2'-(cyclopentane-1,1-diyl)diacetate.

<sup>i</sup> 8-[4-[4-(5-Chloropyrimidin-2-yl)piperazin-1-yl]butyl]-8-azaspiro[4.5]decane-7,9-dione.

<sup>j</sup> 4-(7,9-Dioxo-8-azaspiro[4.5]decan-8-yl)butyl 2-[1-[2-oxo-2-((4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl)amino)ethyl]cyclopentyl]acetate.

#### For impurities detected at UV 210 nm

Calculate the percentage of buspirone related compound K, buspirone related compound L, or buspirone related compound N in the portion of Buspirone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of buspirone related compound K, buspirone related compound L, or buspirone related compound N from the *Sample solution*

$r_S$  = peak response of buspirone related compound K, buspirone related compound L, or buspirone related compound N from the *Standard solution*

$C_S$  = concentration of USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, or USP Buspirone Related Compound N RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Buspirone Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of buspirone bromobutyl analog and any other individual impurity in the portion of Buspirone Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of buspirone bromobutyl analog and any other individual impurity from the *Sample solution*

$r_S$  = peak response of buspirone from the *Standard solution*

$C_S$  = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Buspirone Hydrochloride in the *Sample solution* (mg/mL)

#### Acceptance criteria

**For impurities detected at UV 210 nm:** See Table 3.  
 Disregard any peak below 0.05%.



Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Buspirone related compound K <sup>a</sup>	0.6	0.1
Buspirone	1.0	—
Buspirone related compound L <sup>b</sup>	1.7	0.10
Buspirone bromobutyl analog <sup>c</sup>	1.8	0.10
Buspirone related compound N <sup>d</sup>	1.9	0.10
Any other individual impurity	—	0.10
Total impurities	—	0.2

<sup>a</sup> 8-Azaspiro[4.5]decane-7,9-dione.<sup>b</sup> 8-(4-Chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione.<sup>c</sup> 8-(4-Bromobutyl)-8-azaspiro[4.5]decane-7,9-dione.<sup>d</sup> 8,8'-(Butane-1,4-diyl)bis(8-azaspiro[4.5]decane-7,9-dione).**SPECIFIC TESTS**

- **WATER DETERMINATION**, Method I (921): NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers, at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
  - USP Buspirone Hydrochloride RS
  - USP Buspirone Related Compound A RS  
2-(Piperazin-1-yl)pyrimidine.  
C<sub>8</sub>H<sub>12</sub>N<sub>4</sub> 164.21
  - USP Buspirone Related Compound G RS  
1,4-Di(piperidin-2-yl)piperazine.  
C<sub>12</sub>H<sub>14</sub>N<sub>6</sub> 242.28
  - USP Buspirone Related Compound K RS  
8-Azaspiro[4.5]decane-7,9-dione.  
C<sub>9</sub>H<sub>13</sub>NO<sub>2</sub> 167.21
  - USP Buspirone Related Compound L RS  
8-(4-Chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione.  
C<sub>13</sub>H<sub>20</sub>ClNO<sub>2</sub> 257.76
  - USP Buspirone Related Compound N RS  
8,8'-(Butane-1,4-diyl)bis(8-azaspiro[4.5]decane-7,9-dione).  
C<sub>22</sub>H<sub>32</sub>N<sub>2</sub>O<sub>4</sub> 388.50

**Buspirone Hydrochloride Tablets****DEFINITION**

Buspirone Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of buspirone hydrochloride (C<sub>21</sub>H<sub>31</sub>N<sub>5</sub>O<sub>2</sub> · HCl).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**

**Sample**: Grind 20 Tablets to a fine powder, add 50 mL of chloroform, stir for 3–5 min, and filter into a 250-mL evaporating flask. Evaporate the solution with the aid of a rotary evaporator to dryness at low heat. Use the residue.

**Acceptance criteria**: Meet the requirements

- **B.** The relative retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

- **PROCEDURE**

**Buffer A**: 6.8 g/L of monobasic potassium phosphate and 0.93 g/L of sodium 1-hexanesulfonate monohydrate, adjusted with phosphoric acid to a pH of 3.4

**Buffer B**: 3.4 g/L of monobasic potassium phosphate and 3.52 g/L of sodium 1-hexanesulfonate monohydrate, adjusted with phosphoric acid to a pH of 2.2

**Solution A**: Acetonitrile and Buffer A (5:95)

**Solution B**: Acetonitrile and Buffer B (75:25)

**Mobile phase**: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
6	90	10
34	42	58
45	42	58
55	0	100
56	100	0
60	100	0
61	90	10

**Diluent**: Solution A

**Impurities stock solution**: 0.25 mg/mL each of USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in acetonitrile

**System suitability solution**: 1.0 mg/mL of USP Buspirone Hydrochloride RS and 0.001 mg/mL each of USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in *Diluent* from the *Impurities stock solution*

**Standard solution**: 0.1 mg/mL of USP Buspirone Hydrochloride RS in *Diluent*

**Sample solution**: Nominally 0.1 mg/mL of buspirone hydrochloride from NLT 20 finely powdered Tablets in *Diluent*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 60% of the flask volume of *Diluent*, and sonicate for 30 min. Allow the solution to cool to room temperature, and then dilute with *Diluent* to volume. Centrifuge the solution and filter the supernatant. Further dilute the filtrate with *Diluent* as needed.

**Chromatographic system**

(See Chromatography (621), *System Suitability*.)

**Mode**: LC

**Detector**: UV 240 nm

**Column**: 4.6-mm × 15-cm; 5-μm packing L1

**Column temperature**: 40°

**Flow rate**: 1 mL/min

**Injection volume**: 20 μL

**System suitability**

**Samples**: *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution**: NLT 2.0 between the buspirone and buspirone related compound G peaks, *System suitability solution*

**Tailing factor**: NMT 1.5, *Standard solution*

**Relative standard deviation**: NMT 1.0%, *Standard solution*

**Analysis**

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of buspirone hydrochloride (C<sub>21</sub>H<sub>31</sub>N<sub>5</sub>O<sub>2</sub> · HCl) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)



$C_U$  = nominal concentration of buspirone hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 30 min

**Sample solution:** Filter a portion of the solution under test, and dilute with *Medium* as needed.

**Standard solution:** USP Buspirone Hydrochloride RS in *Medium* having a concentration similar to that expected in the *Sample solution*

### Instrumental conditions

Mode: UV

Analytical wavelength: Maximum at about 235 nm

### Analysis

**Samples:** *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of buspirone hydrochloride ( $C_{21}H_{31}N_5O_2 \cdot HCl$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of buspirone hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 500 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of buspirone hydrochloride ( $C_{21}H_{31}N_5O_2 \cdot HCl$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

**Buffer A, Buffer B, Solution A, Solution B, Mobile phase, Diluent, Impurities stock solution, and System suitability solution:** Proceed as directed in the *Assay*.

**Standard solution:** 0.001 mg/mL each of USP Buspirone Hydrochloride RS, USP Buspirone Related Compound A RS, USP Buspirone Related Compound G RS, USP Buspirone Related Compound K RS, USP Buspirone Related Compound L RS, and USP Buspirone Related Compound N RS in *Diluent*

**Sample solution:** Nominally 1.0 mg/mL of buspirone hydrochloride from NLT 20 finely powdered Tablets in *Diluent*, prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 60% of the flask volume of *Diluent*, and sonicate for 30 min. Allow the solution to cool to room temperature, and then dilute with *Diluent* to volume. Centrifuge the solution and filter the supernatant. Use the filtrate.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 and 240 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 μL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution at 240 nm:** NLT 2.0 between the buspirone and buspirone related compound G peaks, *System suitability solution*

**Resolution at 210 nm:** NLT 4.0 between the buspirone related compound L and buspirone related compound N peaks, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for each peak, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

**For impurities detected at UV 240 nm**

Calculate the percentage of buspirone related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of buspirone related compound A from the *Sample solution*

$r_S$  = peak response of buspirone related compound A from the *Standard solution*

$C_S$  = concentration of USP Buspirone Related Compound A RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of buspirone hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any individual unspecified degradation product from the *Sample solution*

$r_S$  = peak response of buspirone from the *Standard solution*

$C_S$  = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of buspirone hydrochloride in the *Sample solution* (mg/mL)

### Acceptance criteria

**For impurities detected at UV 240 nm:** See *Table 2*. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Buspirone related compound A <sup>a</sup>	0.2	0.20
Spiroammonium salt <sup>b,c</sup>	0.3	—
Bispyrimidinylpiperazinyl butane <sup>c,d</sup>	0.6	—
Bispyrimidinylpiperazinylbutyl ether <sup>c,e</sup>	0.7	—
Buspirone open ring <sup>c,f</sup>	0.8	—
Buspirone open ring dimer <sup>c,g</sup>	0.9	—
Buspirone	1.0	—
Buspirone related compound G <sup>c,h</sup>	1.05	—
Buspirone diester dimer <sup>c,i</sup>	1.1	—
Chlorobuspirone <sup>c,i</sup>	1.2	—
Buspirone open ring spirodimer <sup>c,k</sup>	1.5	—

<sup>a</sup> 2-(Piperazin-1-yl)pyrimidine.

<sup>b</sup> 8-(Pyrimidin-2-yl)-8-aza-5-azoniaspiro[4.5]decane.

<sup>c</sup> Process impurity included for identification only and not included in the calculation of total degradation products.

<sup>d</sup> 1,4-Bis[4-(pyrimidin-2-yl)piperazin-1-yl]butane.

<sup>e</sup> Bis[4-[1-(pyrimidin-2-yl)piperazine-4-yl]butane-1-yl] ether.

<sup>f</sup> 2-[1-[2-Oxo-2-((4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl)amino)ethyl]cyclopentyl]acetic acid.

<sup>g</sup> 4-[4-(Pyrimidin-2-yl)piperazin-1-yl]butyl 2-[1-[2-oxo-2-((4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl)amino)ethyl]cyclopentyl]acetate.

<sup>h</sup> 1,4-Di(piperidin-2-yl)piperazine.

<sup>i</sup> Bis[4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl] 2,2'-(cyclopentane-1,1-diyl)diacetate.

<sup>j</sup> 8-[4-[4-(5-Chloropyrimidin-2-yl)piperazin-1-yl]butyl]-8-azaspiro[4.5]decane-7,9-dione.

<sup>k</sup> 4-(7,9-Dioxo-8-azaspiro[4.5]decan-8-yl)butyl 2-[1-[2-oxo-2-((4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl)amino)ethyl]cyclopentyl]acetate.



Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified degradation product	—	0.2
Total impurities	—	See Table 3

<sup>a</sup> 2-(Piperazin-1-yl)pyrimidine.<sup>b</sup> 8-(Pyrimidin-2-yl)-8-aza-5-azoniaspiro[4.5]decane.<sup>c</sup> Process impurity included for identification only and not included in the calculation of total degradation products.<sup>d</sup> 1,4-Bis[4-(pyrimidin-2-yl)piperazin-1-yl]butane.<sup>e</sup> Bis[4-[1-(pyrimidin-2-yl)piperazine-4-yl]butane-1-yl] ether.<sup>f</sup> 2-[1-[2-Oxo-2-({4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl}amino)ethyl]cyclopentyl]acetic acid.<sup>g</sup> 4-[4-(Pyrimidin-2-yl)piperazin-1-yl]butyl 2-[1-[2-oxo-2-({4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl}amino)ethyl]cyclopentyl]acetate.<sup>h</sup> 1,4-Di(pyrimidin-2-yl)piperazine.<sup>i</sup> Bis[4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl] 2,2'-(cyclopentane-1,1-diyl)diacetate.<sup>j</sup> 8-[4-(5-Chloropyrimidin-2-yl)piperazin-1-yl]butyl]-8-azaspiro[4.5]decane-7,9-dione.<sup>k</sup> 4-(7,9-Dioxo-8-azaspiro[4.5]decan-8-yl)butyl 2-[1-[2-oxo-2-({4-[4-(pyrimidin-2-yl)piperazin-1-yl]butyl}amino)ethyl]cyclopentyl]acetate.**For impurities detected at UV 210 nm**

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of any individual unspecified degradation product from the *Sample solution* $r_S$  = peak response of buspirone from the *Standard solution* $C_S$  = concentration of USP Buspirone Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of buspirone hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria**

For impurities detected at UV 210 nm: See Table 3.

Disregard any peak below 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Buspirone related compound K <sup>a,b</sup>	0.6	—
Buspirone	1.0	—
Buspirone related compound L <sup>b,c</sup>	1.7	—
Buspirone bromobutyl analog <sup>b,d</sup>	1.8	—
Buspirone related compound N <sup>b,e</sup>	1.9	—
Any individual unspecified degradation product	—	0.2
Total impurities	—	2.0 <sup>f</sup>

<sup>a</sup> 8-Azaspiro[4.5]decane-7,9-dione.<sup>b</sup> Process impurity included for identification only and not included in the calculation of total degradation products.<sup>c</sup> 8-(4-Chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione.<sup>d</sup> 8-(4-Bromobutyl)-8-azaspiro[4.5]decane-7,9-dione.<sup>e</sup> 8,8'-(Butane-1,4-diyl)bis(8-azaspiro[4.5]decane-7,9-dione).<sup>f</sup> Total impurities include impurities detected at UV 240 nm.**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at controlled room temperature.

- USP REFERENCE STANDARDS (11)**

USP Buspirone Hydrochloride RS

USP Buspirone Related Compound A RS

2-(Piperazin-1-yl)pyrimidine.

 $C_8H_{12}N_4$  164.21

USP Buspirone Related Compound G RS

1,4-Di(pyrimidin-2-yl)piperazine.

 $C_{12}H_{14}N_6$  242.28

USP Buspirone Related Compound K RS

8-Azaspiro[4.5]decane-7,9-dione.

 $C_9H_{13}NO_2$  167.21

USP Buspirone Related Compound L RS

8-(4-Chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione.

 $C_{13}H_{20}ClNO_2$  257.76

USP Buspirone Related Compound N RS

8,8'-(Butane-1,4-diyl)bis(8-azaspiro[4.5]decane-7,9-dione).

 $C_{22}H_{32}N_2O_4$  388.50**Busulfan** $C_6H_{14}O_6S_2$ 

246.30

1,4-Butanediol, dimethanesulfonate;

1,4-Butanediol dimethanesulfonate [55-98-1].

**DEFINITION**Busulfan contains NLT 98.0% and NMT 100.5% of busulfan ( $C_6H_{14}O_6S_2$ ), calculated on the dried basis.**IDENTIFICATION**

- A.**

**Sample:** 100 mg**Analysis:** Fuse the *Sample* with 100 mg of potassium nitrate and a pellet of potassium hydroxide weighing 250 mg. Cool, dissolve the residue in water, acidify with 3 N hydrochloric acid, and add a few drops of barium chloride TS.**Acceptance criteria:** A white precipitate is formed.

- B.**

**Sample:** 100 mg**Analysis:** Add 10 mL of water and 5 mL of 1 N sodium hydroxide to the *Sample*. Heat until a clear solution is obtained.**Acceptance criteria:** An odor characteristic of methane-sulfonic acid is perceptible.

- C.**

**Sample solution:** Use the solution from the *Analysis* in Identification test B.**Analysis:** Cool the *Sample solution*, and divide it into two equal portions. To the first portion add 1 drop of potassium permanganate TS. Acidify the second portion of the solution with 2 N sulfuric acid, and add 1 drop of potassium permanganate TS.**Acceptance criteria****For first portion:** The purple color changes to violet, then to blue, and finally to emerald-green.**For second portion:** The color of the permanganate is not discharged.**ASSAY**

- PROCEDURE**

**Sample solution:** Transfer 80 mg of Busulfan into a 250-mL conical flask. Add 30 mL of water, swirl, add phenolphthalein TS, and neutralize with 0.05 N sodium hydroxide. Connect the flask to a reflux air condenser, and boil the mixture gently for NLT 30 min, adding water occasionally to maintain the volume. Cool to room temperature.



**Titrimetric system****Mode:** Direct titration**Titrant:** 0.05 N sodium hydroxide VS**Endpoint detection:** Visual**Analysis:** Add phenolphthalein TS to the *Sample solution*, and titrate with *Titrant*. Each mL of *Titrant* is equivalent to 6.158 mg of busulfan ( $C_6H_{14}O_6S_2$ ).**Acceptance criteria:** 98.0%–100.5% on the dried basis**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE** (741): 115°–118°

- **LOSS ON DRYING** (731)

**Analysis:** Dry a sample under vacuum at 60° to constant weight.**Acceptance criteria:** NMT 2.0%**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label bears a warning that great care should be taken to prevent inhaling particles of Busulfan and exposing the skin to it.

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**Busulfan Tablets**

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**DEFINITION**Busulfan Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of busulfan ( $C_6H_{14}O_6S_2$ ).**IDENTIFICATION**• **A.****Sample:** A suitable number of Tablets**Analysis:** Pulverize the *Sample* and extract the powder with several portions of acetone. Evaporate the combined acetone extracts, with the aid of a current of air, on a steam bath.**Acceptance criteria:** The dry residue melts at about 115°.• **B.****Sample:** 100 mg of the powder obtained in *Identification test A***Analysis:** Fuse the *Sample* with 100 mg of potassium nitrate and a pellet of potassium hydroxide weighing 250 mg. Cool, dissolve the residue in water, acidify with 3 N hydrochloric acid, and add a few drops of barium chloride TS.**Acceptance criteria:** A white precipitate is formed.• **C.****Sample:** 100 mg of the powder obtained in *Identification test A***Analysis:** Add 10 mL of water and 5 mL of 1 N sodium hydroxide to the *Sample*. Heat until a clear solution is obtained.**Acceptance criteria:** An odor characteristic of methane-sulfonic acid is perceptible.• **D.****Sample solution:** Use the solution from *Identification test C*.**Analysis:** Cool the *Sample solution*, and divide it into two equal portions. To the first portion add 1 drop of potassium permanganate TS. Acidify the second portion of the solution with 2 N sulfuric acid, and add 1 drop of potassium permanganate TS.**Acceptance criteria****For first portion:** The purple color changes to violet, then to blue, and finally to emerald-green.**For second portion:** The color of the permanganate is not discharged.**ASSAY**• **PROCEDURE**

Guard against accidental inhalation of the fine powder.

**Sample solution:** Transfer an equivalent to 80 mg of busulfan, from finely powdered Tablets (NLT 40), to a 100-mL beaker. Extract with four 20-mL portions of acetone, each time stirring the mixture well. Allow the insoluble matter to settle, and decant the supernatant through a sintered-glass filter into a 250-mL conical flask. Evaporate the combined acetone extracts to about 10 mL, add phenolphthalein TS, and neutralize with 0.05 N sodium hydroxide. Evaporate to dryness, and add about 30 mL of water. Connect the flask to a reflux air condenser, and boil the mixture gently for NLT 30 min, adding water occasionally to maintain the volume. Cool to room temperature.**Titrimetric system****Mode:** Direct titration**Titrant:** 0.05 N sodium hydroxide VS**Endpoint detection:** Visual**Analysis:** Add phenolphthalein TS to the *Sample solution*, and titrate with *Titrant*. Each mL of *Titrant* is equivalent to 6.158 mg of the labeled amount of busulfan ( $C_6H_{14}O_6S_2$ ).**Acceptance criteria:** 93.0%–107.0%**PERFORMANCE TESTS**

- **DISINTEGRATION** (701)

Time: 30 min, the use of disks being omitted

**Acceptance criteria:** Meet the requirements

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

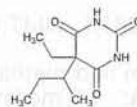
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

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**Butabarbital**

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 $C_{10}H_{16}N_2O_3$ 

2,4,6-(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-methylpropyl)-;

5-sec-Butyl-5-ethylbarbituric acid [125-40-6].

212.25

**DEFINITION**Butabarbital contains NLT 98.5% and NMT 101.0% of butabarbital ( $C_{10}H_{16}N_2O_3$ ), calculated on the dried basis.**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197M)

**ASSAY**• **PROCEDURE****Internal standard solution:** 2 mg/mL of tetracosane in chloroform**Standard stock solution:** 2 mg/mL of USP Butabarbital RS in chloroform**Standard solution:** 1 mg/mL of USP Butabarbital RS from *Standard stock solution* in *Internal standard solution* prepared as follows. Combine 10.0 mL of *Standard stock solution* and 10.0 mL of *Internal standard solution*.**Sample stock solution:** 2 mg/mL of Butabarbital in chloroform**Sample solution:** 1 mg/mL of Butabarbital from *Sample stock solution* in *Internal standard solution* prepared as



follows. Combine 10.0 mL of *Sample stock solution* and 10.0 mL of *Internal standard solution*.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 4-mm × 1.8-m; 10% phase G37 on support S1AB

Temperatures

Injection port: 260°

Detector: 300°

Column: 260°

Carrier gas: Dry nitrogen

Flow rate: 50 mL/min

Injection volume: 2 µL

#### System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for butabarbital and tetracosane are 0.6 and 1.0, respectively.]

#### Suitability requirements

Resolution: NLT 3.0 between butabarbital and tetracosane

Tailing factor: NMT 1.3 for butabarbital and NMT 1.2 for tetracosane

Relative standard deviation: NMT 1.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of butabarbital ( $C_{10}H_{15}N_2O_3$ ) in the portion of Butabarbital taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of butabarbital to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of butabarbital to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Butabarbital RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Butabarbital in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the dried basis

#### IMPURITIES

• **RESIDUE ON IGNITION** <281>: NMT 0.1%

#### • ORGANIC IMPURITIES

Diluent: Chloroform and methanol (50:50)

Standard solution A: 4.0 mg/mL of USP Butabarbital RS in *Diluent*

Standard solution B: 0.4 mg/mL of USP Butabarbital RS from *Standard solution A* in *Diluent*

Sample solution: 40 mg/mL of Butabarbital in *Diluent*

#### Chromatographic system

(See *Chromatography* <621>, *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system: Acetone, methylene chloride, methanol, and ammonium hydroxide (5:3:1:1)

Spray reagent: A solution of mercurous nitrate dihydrate in 0.15 N nitric acid (1 in 100)

#### Analysis

Samples: *Standard solution A*, *Standard solution B*, and *Sample solution*

Proceed as directed in the chapter. Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and dry under a current of air. Spray the plate with *Spray reagent*, and immediately estimate the intensities of any spots of the *Sample solution*, other than the principal spot, in comparison with *Standard solution B*.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*; and the sum of the intensities of any secondary spots of the *Sample solution* is NMT the intensity of the principal spot produced by *Standard solution B*, corresponding to NMT a total of 1% of impurities.

#### SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE**, *Class Ia* <741>: 164°–167°

• **LOSS ON DRYING** <731>

Analysis: Dry at 105° for 2 h.

Acceptance criteria: NMT 1.0%

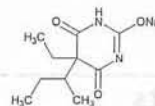
#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Butabarbital RS

## Butabarbital Sodium



$C_{10}H_{15}N_2NaO_3$  234.23  
2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-methylpropyl)-, monosodium salt;  
Sodium 5-sec-butyl-5-ethylbarbiturate [143-81-7].

#### DEFINITION

Butabarbital Sodium contains NLT 98.2% and NMT 100.5% of butabarbital sodium ( $C_{10}H_{15}N_2NaO_3$ ), calculated on the dried basis.

#### IDENTIFICATION

• **A. INFRARED ABSORPTION** <197K>

Sample: 150 mg of Butabarbital Sodium

Analysis: Transfer the *Sample* to a suitable separator, dissolve in 10 mL of water, and add 15 mL of 3 N hydrochloric acid. Extract with three 20-mL portions of chloroform, filter the extracts through anhydrous sodium sulfate, and collect the extracts in a suitable beaker. Evaporate the combined extracts on a steam bath with the aid of a current of air to dryness, and dry the residue at 105° for 2 h.

Acceptance criteria: Meets the requirements

• **B. ULTRAVIOLET ABSORPTION** <197U>

Analytical wavelength: 240 nm

Buffer: pH 9.6 alkaline borate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)

Standard solution: 10 µg/mL of USP Butabarbital RS in *Buffer*

Sample solution: 10 µg/mL of Butabarbital Sodium in *Buffer*

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

• **C. IDENTIFICATION TESTS—GENERAL**, *Sodium* <191>

Sample: 100 mg of Butabarbital Sodium

Analysis: Ignite the *Sample*, and proceed as directed in the chapter using the residue.

Acceptance criteria: Meets the requirements

#### ASSAY

• **PROCEDURE**

Buffer: pH 9.6 alkaline borate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)

Standard stock solution: 0.125 mg/mL of USP Butabarbital RS in *Buffer*



**Standard solution:** 0.0125 mg/mL of USP Butabarbital RS from *Standard stock solution in Buffer*

**Sample stock solution:** 0.140 mg/mL of Butabarbital Sodium in *Buffer*

**Sample solution:** 0.0140 mg/mL from *Sample stock solution in Buffer*

#### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** Maximum absorbance at about 240 nm

**Blank:** *Buffer*

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Concomitantly determine the absorbances of the solutions.

Calculate the percentage of butabarbital sodium ( $C_{10}H_{15}N_2NaO_3$ ) in the portion of Butabarbital Sodium taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Butabarbital RS in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = concentration of Butabarbital Sodium in the *Sample solution* ( $\mu\text{g/mL}$ )

$M_{r1}$  = molecular weight of butabarbital sodium, 234.23

$M_{r2}$  = molecular weight of butabarbital, 212.25

**Acceptance criteria:** 98.2%–100.5% on the dried basis

#### IMPURITIES

##### Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 30 ppm (Official 1-Jan-2018)

##### • ORGANIC IMPURITIES

**Diluent:** Chloroform and methanol (50:50)

**Standard solution A:** 4.0 mg/mL of USP Butabarbital RS in *Diluent*

**Standard solution B:** 0.4 mg/mL of USP Butabarbital RS from *Standard solution A* in *Diluent*

**Sample solution:** 44 mg/mL of Butabarbital Sodium in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10  $\mu\text{L}$

**Developing solvent system:** Acetone, methylene chloride, methanol, and ammonium hydroxide (5:3:1:1)

**Spray reagent:** A solution of mercurous nitrate dihydrate in 0.15 N nitric acid (1 in 100)

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and dry under a current of air. Spray the plate with *Spray reagent*, and immediately estimate the intensities of any spots of the *Sample solution*, other than the principal spot, in comparison with *Standard solution B*.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*; and the sum of the intensities of any secondary spots of the *Sample solution* is NMT the intensity of the principal spot produced by *Standard solution B*, corresponding to NMT a total of 1% of impurities.

#### SPECIFIC TESTS

##### • COMPLETENESS OF SOLUTION

**Sample solution:** Dissolve 1.0 g of Butabarbital Sodium in 10 mL of carbon dioxide-free water.

**Acceptance criteria:** After 1 min, the solution is clear and free from undissolved solid.

##### • pH (791)

**Sample solution:** Use the *Sample solution* from the test for *Completeness of Solution*.

**Acceptance criteria:** 10.0–11.2

##### • LOSS ON DRYING (731)

**Analysis:** Dry at 150° to constant weight.

**Acceptance criteria:** NMT 5.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**  
USP Butabarbital RS

## Butabarbital Sodium Oral Solution

#### DEFINITION

Butabarbital Sodium Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of butabarbital sodium ( $C_{10}H_{15}N_2NaO_3$ ).

#### IDENTIFICATION

##### • A. INFRARED ABSORPTION (197K)

**Sample:** Transfer an equivalent to 150 mg of butabarbital sodium from a volume of Oral Solution, to a separator. Render it distinctly alkaline by the addition of 1 N sodium hydroxide, and saturate it with sodium chloride. Extract the mixture with two 15-mL portions of ether, and discard the ether. Acidify the solution with hydrochloric acid, and render it just alkaline to litmus by adding small portions of sodium bicarbonate (carbonate-free). Extract the liberated acid barbiturate using five 20-mL portions of chloroform. Wash the combined chloroform extracts with 10 mL of water acidified with 1 drop of hydrochloric acid, then extract the water with 10 mL of chloroform, adding the latter to the main chloroform solution. Pass the chloroform solution through a pledget of cotton or other suitable filter, previously washed with chloroform, into a tared beaker, and finally wash the separator and the filter with three 5-mL portions of chloroform. Evaporate the combined chloroform solution and washings on a steam bath with the aid of a current of air to dryness, and dry the residue at 105° for 2 h.

**Acceptance criteria:** Meets the requirements

- **B.** The retention time of the butabarbital peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Solution A:** Dissolve 2.0 mL of bromine and 10 g of potassium bromide in 60 mL of water.

**Solution B:** Sodium metabisulfite in water (1 in 10)

**Internal standard solution:** 0.7 mg/mL of secobarbital in chloroform

**Standard solution:** 1 mg/mL of USP Butabarbital RS and 1.4 mg/mL of secobarbital in chloroform

**Sample stock solution:** Nominally 0.3 mg/mL of butabarbital sodium from Oral Solution prepared as follows. Transfer a volume of Oral Solution, equivalent to 30 mg of butabarbital sodium, to a separator. Add 1 mL of *Solution A*, and swirl. Allow to stand for 5 min, add 1 mL of *Solution B*, and swirl. Add 300 mg of sodium bicarbonate in small portions, with mixing, and extract with four 10-mL portions of chloroform. Pass the extracts through about 15 g of anhydrous sodium sulfate that is



supported on a funnel by a small pledget of glass wool. Collect the combined filtrates in a 50-mL volumetric flask, wash the sodium sulfate with 5 mL of chloroform, collecting the washing with the filtrate, dilute with chloroform to volume, and mix.

[NOTE—This solution includes a bromination step for elimination of parabens and a carbonate-chloroform extraction for elimination of benzoic acid.]

**Sample solution:** Combine 2.0 mL of *Sample stock solution* with 2.0 mL of *Internal standard solution* in a suitable container, and reduce the volume to about 1 mL by evaporation, with the aid of a stream of dry nitrogen, at room temperature.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 4-mm × 0.9-m glass; packed with 3% liquid phase G10 support on 80- to 10-mesh S1A

**Temperatures**

**Injection port:** 225°

**Detector:** 225°

**Column:** 200 ± 10°

**Carrier gas:** A suitable gas such as dry nitrogen

**Flow rate:** 60–80 mL/min

**Injection volume:** 5 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for butabarbital and secobarbital are 0.6 and 1.0, respectively.]

**Resolution:** NLT 2.4 between butabarbital and secobarbital

**Tailing factor:** NMT 2.0 each for butabarbital and secobarbital

**Relative standard deviation:** NMT 1.5% for the peak response ratio of butabarbital to the internal standard

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of butabarbital sodium ( $C_{10}H_{15}N_2NaO_3$ ) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak response ratio of butabarbital to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of butabarbital to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Butabarbital RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of butabarbital sodium in the *Sample solution* (µg/mL)

$M_{r1}$  = molecular weight of butabarbital sodium, 234.23

$M_{r2}$  = molecular weight of butabarbital, 212.25

**Acceptance criteria:** 90.0%–110.0%

#### OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method II (611):** Between 95.0% and 115.0% of the labeled amount of alcohol ( $C_2H_5OH$ )

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

#### • USP REFERENCE STANDARDS (11)

USP Butabarbital RS

### Butabarbital Sodium Tablets

#### DEFINITION

Butabarbital Sodium Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of butabarbital sodium ( $C_{10}H_{15}N_2NaO_3$ ).

#### IDENTIFICATION

- **A.** The retention time of the butabarbital peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Solution A:** Ammonium hydroxide in water (1 in 25)  
**Internal standard solution:** 1.2 mg/mL of secobarbital in chloroform

**Standard solution:** 0.8 mg/mL of USP Butabarbital RS and 1 mg/mL of secobarbital in chloroform

**Sample stock solution:** Finely powder NLT 20 Tablets.

Transfer a portion of the powder, equivalent to 50 mg of butabarbital sodium, to a 50-mL volumetric flask.

Add 35 mL of *Solution A*, and dilute with water to volume. Filter, if necessary, discarding the first 15 mL of the filtrate, and transfer 25.0 mL of the clear solution to a separator. Add 2 mL of hydrochloric acid, and extract with three 25-mL portions of chloroform. Filter the extracts through about 15 g of anhydrous sodium sulfate that is supported on a funnel by a small pledget of glass wool. Collect the combined filtrate in a 100-mL volumetric flask, and wash the sodium sulfate with 15 mL of chloroform, collecting the washing with the filtrate. Dilute with chloroform to volume.

**Sample solution:** Combine 4.0 mL of *Sample stock solution* with 1.0 mL of *Internal standard solution* in a suitable container. Reduce the volume to about 1 mL by evaporation, with the aid of a stream of nitrogen, at room temperature.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 4-mm × 0.9-m glass; packed with 3% liquid phase G10 support on 80- to 10-mesh S1A

**Temperatures**

**Injection port:** 225°

**Detector:** 225°

**Column:** 200 ± 10°

**Carrier gas:** A suitable gas such as dry nitrogen

**Flow rate:** 60–80 mL/min

**Injection volume:** 5 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for butabarbital and secobarbital are 0.6 and 1.0, respectively.]

**Resolution:** NLT 2.4 between butabarbital and secobarbital

**Tailing factor:** NMT 2.0 each for butabarbital and secobarbital

**Relative standard deviation:** NMT 1.5% for the peak response ratio of butabarbital to the internal standard

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of butabarbital sodium ( $C_{10}H_{15}N_2NaO_3$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$



- $R_U$  = peak response ratio of butabarbital to the internal standard from the *Sample solution*  
 $R_S$  = peak response ratio of butabarbital to the internal standard from the *Standard solution*  
 $C_S$  = concentration of USP Butabarbital RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of butabarbital sodium in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of butabarbital sodium, 234.23  
 $M_{r2}$  = molecular weight of butabarbital, 212.25  
 Acceptance criteria: 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 45 min

Standard solution: USP Butabarbital RS in Medium

Sample solution: Pass a portion of the solution under test through a suitable filter, and mix with sufficient ammonium hydroxide to provide a concentration of 0.5 N ammonium hydroxide. Dilute with Medium, if necessary.

#### Instrumental conditions

Mode: UV

Analytical wavelength: 239 nm

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of butabarbital sodium ( $C_{10}H_{15}N_2NaO_3$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times D \times (M_{r1}/M_{r2}) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Butabarbital RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of butabarbital sodium in the *Sample solution* (mg/mL)

$D$  = dilution factor for the *Sample solution*

$M_{r1}$  = molecular weight of butabarbital sodium, 234.23

$M_{r2}$  = molecular weight of butabarbital, 212.25

Tolerances: NLT 75% (Q) of the labeled amount of butabarbital sodium ( $C_{10}H_{15}N_2NaO_3$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

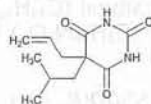
### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

#### • USP REFERENCE STANDARDS (11)

USP Butabarbital RS

## Butalbital



$C_{11}H_{16}N_2O_3$  224.26  
 2,4,6-(1*H*,3*H*,5*H*)-Pyrimidinetrione, 5-(2-methylpropyl)-5-(2-propenyl)-;  
 5-Allyl-5-isobutylbarbituric acid [77-26-9].

### DEFINITION

Butalbital contains NLT 98.0% and NMT 102.0% of butalbital ( $C_{11}H_{16}N_2O_3$ ), calculated on the dried basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

Buffer: 1.0 mL of phosphoric acid diluted with water to 1 L

Mobile phase: Acetonitrile and Buffer (25:75)

System suitability solution: 0.1 mg/mL each of USP Butalbital RS and USP Salicylic Acid RS in *Mobile phase*. Sonication may be used to aid in dissolution.

Standard solution: 0.1 mg/mL of USP Butalbital RS in *Mobile phase*. Sonication may be used to aid in dissolution.

Sample solution: 0.1 mg/mL of Butalbital in *Mobile phase*. Sonication may be used to aid in dissolution.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 10-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μL

#### System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times of salicylic acid and butalbital are 0.86 and 1.0, respectively.]

#### Suitability requirements

Resolution: NLT 3.0 between salicylic acid and butalbital, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of butalbital ( $C_{11}H_{16}N_2O_3$ ) in the portion of Butalbital taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Butalbital RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Butalbital in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

#### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1: Jan-2018)

#### • ORGANIC IMPURITIES

Buffer: 4.1 g/L of monobasic potassium phosphate adjusted with 1 N sodium hydroxide to a pH of 6.0

Mobile phase: Acetonitrile and Buffer (22:78)

System suitability solution: 10 μg/mL each of USP Butalbital RS and USP Butabarbital RS in *Mobile phase*. Sonication may be used to aid in dissolution.

Sample solution: 1 mg/mL of Butalbital in *Mobile phase*. Sonication may be used to aid in dissolution.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; 5-μm packing L78

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μL

**System suitability**Sample: *System suitability solution*

[NOTE—The relative retention times of butabarbital and butalbital are 0.83 and 1.0, respectively.]

**Suitability requirements**

Resolution: NLT 2.0 between butabarbital and butalbital

Tailing factor: NMT 1.5 for butalbital

Relative standard deviation: NMT 5.0% for butalbital

**Analysis**Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Butalbital taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution* $r_T$  = sum of the peak responses from the *Sample solution***Acceptance criteria**

Any individual unspecified impurity: NMT 0.10%

Total impurities: NMT 1%

**SPECIFIC TESTS****• Loss on Drying (731)**

Analysis: Dry under vacuum at room temperature to constant weight.

Acceptance criteria: NMT 0.2%

**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers.**• USP REFERENCE STANDARDS (11)**

USP Butabarbital RS

USP Butalbital RS

USP Salicylic Acid RS

**Butalbital, Acetaminophen, and Caffeine Capsules**

» Butalbital, Acetaminophen, and Caffeine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), acetaminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ).

**Packaging and storage**—Preserve in tight containers.**USP Reference standards (11)**

USP Acetaminophen RS

USP Butalbital RS

USP Caffeine RS

**Identification**—The retention times of the butalbital, acetaminophen, and caffeine peaks in the chromatogram of the *Assay preparation* correspond to those of the butalbital, acetaminophen, and caffeine peaks in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution (711)**

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 60 minutes.

**Mobile phase and Chromatographic system**—Prepare as directed in the *Assay* under *Butalbital, Acetaminophen, and Caffeine Tablets*.

**Standard preparation**—Prepare a solution in methanol having known concentrations of about 0.02A mg of USP Acetaminophen RS per mL, 0.02B mg of USP Butalbital RS per mL, and 0.02C mg of USP Caffeine RS per mL, in which A, B, and C are the labeled amounts, in mg of acetaminophen, butalbital, and caffeine, respectively, per Capsule. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Pass a portion of the solution under test through a filter of 10-μm or finer porosity. Separately inject equal volumes (about 20 μL) of the filtrate and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of butalbital ( $C_{11}H_{16}N_2O_3$ ), acetaminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) dissolved by the same formula:

$$900C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the solution under test and the *Standard preparation*, respectively.

**Tolerances**—Not less than 80% (Q) of the labeled amounts of  $C_{11}H_{16}N_2O_3$ ,  $C_8H_9NO_2$ , and  $C_8H_{10}N_4O_2$  is dissolved in 60 minutes.

**Uniformity of dosage units (905):** meet the requirements.

**Assay**

**Mobile phase, Internal standard solution, Butalbital standard stock solution, Caffeine standard stock solution, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay* under *Butalbital, Acetaminophen, and Caffeine Tablets*.

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about the weight of the contents of 1 Capsule, to a 200-mL volumetric flask, add *Internal standard solution* to volume, and mix. Sonicate for 15 minutes, mix, and allow to cool and settle. Transfer 20.0 mL of the clear supernatant to a 50-mL volumetric flask, dilute with water to volume, and mix. Pass a portion of this solution through a filter of 0.5 μm or finer porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the major peaks. Calculate the quantities, in mg, of butalbital ( $C_{11}H_{16}N_2O_3$ ), acetaminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) in the portion of Capsules taken by the formula:

$$500D(R_U/R_S)$$

in which D is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the corresponding analyte to phenacetin obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Butalbital, Acetaminophen, and Caffeine Tablets

» Butalbital, Acetaminophen, and Caffeine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), acetaminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Acetaminophen RS

USP Butalbital RS

USP Caffeine RS

**Identification**—The retention times of the butalbital peak, the acetaminophen peak, and the caffeine peak in the chromatogram of the *Assay preparation* correspond to those of the butalbital peak, the acetaminophen peak, and the caffeine peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution**, *Procedure for a Pooled Sample* (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 30 minutes.

**Mobile phase and Chromatographic system**—Prepare as directed in the *Assay*.

**Standard preparation**—Prepare a solution in methanol having known concentrations of about 0.02A mg of USP Acetaminophen RS per mL, 0.02B mg of USP Butalbital RS per mL, and 0.02C mg of USP Caffeine RS per mL, in which A, B, and C are the labeled amounts, in mg, of acetaminophen, butalbital, and caffeine, respectively, per Tablet. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Pass a portion of the solution under test through a suitable filter having a 10- $\mu$ m or finer porosity. Separately inject equal volumes (about 20  $\mu$ L) of the filtrate and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg, of butalbital ( $C_{11}H_{16}N_2O_3$ ), acetaminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) dissolved by the same formula:

$$900C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of the corresponding analyte obtained from the solution under test and the *Standard preparation*, respectively.

**Tolerances**—Not less than 80% (Q) of the labeled amounts of  $C_{11}H_{16}N_2O_3$ ,  $C_8H_9NO_2$ , and  $C_8H_{10}N_4O_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Mobile phase**—Transfer 800 mg of monobasic potassium phosphate to a 2000-mL volumetric flask. Dissolve in 1100 mL of water, dilute with methanol to volume, and mix. Pass through a suitable filter having a 0.5- $\mu$ m or finer porosity. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of phenacetin in methanol containing 0.65 mg per mL.

**Butalbital standard stock solution**—Dissolve an accurately weighed quantity of USP Butalbital RS in *Internal standard solution* to obtain a solution having a known concentration of about 0.01B mg per mL, B being the labeled amount, in

mg, of butalbital per Tablet, sonicating and shaking the solution, if necessary, to achieve complete dissolution.

**Caffeine standard stock solution**—Dissolve an accurately weighed quantity of USP Caffeine RS in *Internal standard solution* to obtain a solution having a known concentration of about 0.01C mg per mL, C being the labeled amount, in mg, of caffeine per Tablet, sonicating and shaking the solution, if necessary, to achieve complete dissolution.

**Standard preparation**—Transfer to a 50-mL volumetric flask about 0.1A mg of USP Acetaminophen RS, A being the labeled amount, in mg, of acetaminophen per Tablet, 10.0 mL of *Butalbital standard stock solution*, and 10.0 mL of *Caffeine standard stock solution*, sonicate for 5 minutes, dilute with water to volume, and mix. This solution contains about 0.002B mg of butalbital, 0.002A mg of acetaminophen, and 0.002C mg of caffeine per mL. Pass a portion of this solution through a suitable filter having a 0.5- $\mu$ m or finer porosity, and use the filtrate as the *Standard preparation*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 1 average Tablet weight, to a 200-mL volumetric flask, add *Internal standard solution* to volume, and mix. Sonicate for 15 minutes, mix, and allow to cool and settle. Transfer 20.0 mL of the clear supernatant to a 50-mL volumetric flask, dilute with water to volume, and mix. Pass a portion of this solution through a suitable filter having a 0.5- $\mu$ m or finer porosity, discarding the first 5 mL of the filtrate. Use the clear filtrate as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 216-nm detector and a 4-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.16 for acetaminophen, 0.33 for caffeine, 0.77 for phenacetin, and 1.0 for butalbital; the resolution, R, between any two peaks is not less than 1.2; the column efficiency, calculated from the butalbital peak, is not less than 1000 theoretical plates; and the relative standard deviations of the acetaminophen, caffeine, and butalbital responses for replicate injections are not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for the major peaks. Calculate the quantities, in mg, of butalbital ( $C_{11}H_{16}N_2O_3$ ), acetaminophen ( $C_8H_9NO_2$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) in the portion of Tablets taken by the same formula:

$$500D(R_U / R_S)$$

in which D is the concentration, in mg per mL, of the appropriate USP Reference Standard in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the corresponding analyte to phenacetin obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Butalbital and Aspirin Tablets

### DEFINITION

Butalbital and Aspirin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ) and aspirin ( $C_9H_8O_4$ ).

### IDENTIFICATION

- **A.** The retention times of the butalbital and aspirin peaks of the *Sample solution* correspond to that of the butalbital peak of *Standard solution A* and the aspirin peak of *Standard solution B*, as obtained in the *Assay*.



**ASSAY****• PROCEDURE**

**Mobile phase:** Acetonitrile, water, and phosphoric acid (725:3100:4)

**Diluent:** Add 10 mL of formic acid to each L of acetonitrile.

**System suitability solution:** 260 µg/mL of USP Butalbital RS and 12 µg/mL of USP Salicylic Acid RS in *Diluent*, where *J* is the ratio of the labeled amount of butalbital, in mg/Tablet, relative to the labeled amount of aspirin, in mg/Tablet

**Standard solution A:** 325 µg/mL of USP Butalbital RS and 2.4 µg/mL of USP Salicylic Acid RS in *Diluent*, where *J* is the ratio of the labeled amount of butalbital, in mg/Tablet, relative to the labeled amount of aspirin, in mg/Tablet

**Standard solution B:** 325 µg/mL of USP Aspirin RS in *Diluent*

**Sample solution:** Nominally 320 µg/mL of aspirin from a suitable amount of powdered Tablets in solution prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer a portion of this fine powder to a suitable volumetric flask. Dilute with *Diluent* to volume, and sonicate for 15 min. Pass a portion of the solution through a filter of 0.5-µm pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 3.9-mm × 30-cm; 10-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 µL

**System suitability**

**Samples:** *System suitability solution*, *Standard solution A*, and *Standard solution B*

[NOTE—The relative retention times for aspirin, salicylic acid, and butalbital are about 0.6, 0.85, and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.0 between salicylic acid and butalbital, *System suitability solution*

**Relative standard deviation**

**Standard solution A:** NMT 3.0% for butalbital; NMT 6.0% for salicylic acid

**Standard solution B:** NMT 3.0% for aspirin

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Calculate the percentage of butalbital ( $C_{11}H_{16}N_2O_3$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of butalbital from the *Sample solution*

$r_s$  = peak response of butalbital from *Standard solution A*

$C_s$  = concentration of USP Butalbital RS in *Standard solution A* (µg/mL)

$C_u$  = nominal concentration of butalbital in the *Sample solution* (µg/mL)

Calculate the percentage of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of aspirin from the *Sample solution*

$r_s$  = peak response of aspirin from *Standard solution B*

$C_s$  = concentration of USP Aspirin RS in *Standard solution B* (µg/mL)

$C_u$  = nominal concentration of aspirin in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ) and aspirin ( $C_9H_8O_4$ )

**PERFORMANCE TESTS****• DISSOLUTION (711)**

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 60 min

**Butalbital**

**Mobile phase:** Acetonitrile, water, and phosphoric acid (725:3100:4).

**Standard solution:** *L* µg/mL of USP Butalbital RS and 30 µg/mL of salicylic acid in *Mobile phase*, where *L* is the labeled amount of butalbital, in mg/Tablet

**Sample solution:** Use portions of the solution under test passed through a suitable filter of 0.5-µm pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 3.9-mm × 30-cm; 10-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10–25 µL; equal volumes of the *Standard solution* and *Sample solution*

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for aspirin, salicylic acid, and butalbital are about 0.6, 0.85, and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.0 between salicylic acid and butalbital

**Relative standard deviation:** NMT 3.0% for butalbital

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of butalbital ( $C_{11}H_{16}N_2O_3$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times (1/L) \times 100$$

$r_u$  = peak response of butalbital from the *Sample solution*

$r_s$  = peak response of butalbital from the *Standard solution*

$C_s$  = concentration of USP Butalbital RS in the *Standard solution* (µg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim of butalbital (mg/Tablet)

**Aspirin**

**Buffer:** Dissolve 5.98 g of sodium acetate trihydrate in 500 mL of water, add 2.5 mL of glacial acetic acid, dilute with water to 1000 mL, and adjust with glacial acetic acid to a pH of 4.5.

**Sample solution:** Use a filtered portion of the solution under test diluted with 4 volumes of *Buffer*.

**Standard solution:** A known concentration of USP Aspirin RS in water diluted with 4 volumes of *Buffer*. Prepare the *Standard solution* at the time of use.

[NOTE—An amount of alcohol not to exceed 1% of the total volume of the *Standard solution* may be used to bring the Reference Standard into solution before dilution first with water and then with 4 volumes of *Buffer*.]

**Instrumental conditions**

**Mode:** UV-Vis

**Analytical wavelength:** The isosbestic point of aspirin and salicylic acid at  $265 \pm 2$  nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of aspirin ( $C_9H_8O_4$ ) dissolved.



**Tolerances:** NLT 75% (Q) of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ) and aspirin ( $C_9H_8O_4$ ) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905)**

**Analysis:** Proceed as directed in the chapter.

**Acceptance criteria**

**Butalbital:** Meet the requirements for *Content Uniformity*

**Aspirin:** Meet the requirements for *Weight Variation*

**IMPURITIES**

• **LIMIT OF FREE SALICYLIC ACID**

**Mobile phase, System suitability solution, Standard solution A, Standard solution B, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Samples:** *Standard solution A* and *Sample solution*  
Calculate the percentage of free salicylic acid in the Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of salicylic acid from the *Sample solution*

$r_S$  = peak response of salicylic acid from *Standard solution A*

$C_S$  = concentration of USP Salicylic Acid RS in *Standard solution A* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of aspirin in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** NMT 3.0% of free salicylic acid

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Aspirin RS  
USP Butalbital RS  
USP Salicylic Acid RS

## Butalbital, Aspirin, and Caffeine Capsules

**DEFINITION**

Butalbital, Aspirin, and Caffeine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ).

**IDENTIFICATION**

• **A.** The retention times of the butalbital, aspirin, and caffeine peaks of the *Sample solution* correspond to those of the butalbital, aspirin, and caffeine peaks of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

• **PROCEDURE**

**Buffer:** 1.36 g/L of monobasic potassium phosphate in water

**Mobile phase:** Methanol and *Buffer* (45:55) initially adjusted with phosphoric acid to a pH of 3.9. If the retention time of the salicylic acid peak differs from that of the aspirin peak, adjust the pH of the *Mobile phase* with 0.2 N potassium hydroxide or 1 M phosphoric acid so that the salicylic acid peak has the same retention time as that of the aspirin peak. [NOTE—The retention time of the salicylic acid peak decreases about 0.3 min for each 0.1 pH increase. The retention time of the aspirin peak is essentially unaffected by such pH adjustments.]

**Diluent:** Methanol and *Buffer* (45:55) adjusted with phosphoric acid to a pH of  $2.5 \pm 0.05$ .

**Salicylic acid solution:** 0.1 mg/mL of salicylic acid in *Diluent*. Pass this solution through a suitable filter of 0.5- $\mu\text{m}$  or finer pore size.

**Standard stock solution:** 1.6 mg/mL of USP Aspirin RS in *Diluent*. Sonication and shaking may be used to promote dissolution. Use this solution within 24 h.

**Standard solution:** USP Reference Standards in *Standard stock solution* as listed below. Sonication and shaking the solution may be used to promote dissolution. Use this solution within 24 h.

**Butalbital:** 1.6/ $j$  mg/mL of USP Butalbital RS, where  $j$  is the ratio of the labeled amount, in mg, of butalbital relative to the labeled amount of aspirin in mg/  
Capsule

**Caffeine:** 1.6/ $j'$  mg/mL of USP Caffeine RS, where  $j'$  is the ratio of the labeled amount, in mg, of caffeine relative to the labeled amount of aspirin in mg/  
Capsule

**Sample solution:** Nominally 1.6 mg/mL of aspirin from the contents of Capsules in solution prepared as follows. Transfer a suitable portion of the contents of NLT 20 Capsules to an appropriate volumetric flask. Dilute with *Diluent* to volume, and sonicate for 30 min. Pass a portion of this solution through a suitable filter of 0.5- $\mu\text{m}$  or finer pore size. Use the filtrate within 24 h.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detectors**

**Butalbital:** UV 210 nm

**Aspirin and caffeine:** UV at the wavelength of the isosbestic point of aspirin and salicylic acid at about 277 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Column temperature:**  $35 \pm 1^\circ$

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu\text{L}$

**System suitability**

**Samples:** *Salicylic acid solution* and *Standard solution*  
[NOTE—The relative retention times for caffeine, aspirin, salicylic acid, and butalbital are about 0.45, 0.6, 0.6, and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between caffeine and aspirin, *Standard solution*

**Column efficiency:** NLT 2000 theoretical plates from butalbital, *Standard solution*

**Relative standard deviation:** NMT 2.0% each for caffeine, aspirin, and butalbital responses, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ) and caffeine ( $C_8H_{10}N_4O_2$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of butalbital or caffeine from the *Sample solution*

$r_S$  = peak response of butalbital or caffeine from the *Standard solution*

$C_S$  = concentration of USP Butalbital RS or USP Caffeine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of butalbital or caffeine in the *Sample solution* (mg/mL)

Determine the amount, in mg, of aspirin and salicylic acid in the portion of Capsules taken (W):

$$\text{Result} = (r_U/r_S) \times C_S \times V$$

$r_U$  = peak response of aspirin and salicylic acid from the *Sample solution*

$r_S$  = peak response of aspirin and salicylic acid from the *Standard solution*



$C_s$  = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)

$V$  = volume of the *Sample solution* (mL)

Calculate the percentage of the labeled amount of aspirin ( $C_9H_8O_4$ ) in the portion of Capsules taken:

$$\text{Result} = \{W - [(F/100) \times W]\} / (C_u \times V) \times 100$$

$W$  = amount of aspirin and salicylic acid in the portion of Capsules taken to prepare the *Sample solution* (mg)

$F$  = percentage of salicylic acid obtained in the *Limit of Free Salicylic Acid* procedure (%)

$C_u$  = nominal concentration of aspirin in the *Sample solution* (mg/mL)

$V$  = volume of the *Sample solution* (mL)

Acceptance criteria: 90.0%–110.0% each of butalbital, aspirin, and caffeine

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: Water; 1000 mL

Apparatus 2: 50 rpm

Time: 60 min

Buffer, Mobile phase, Diluent, Salicylic acid solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Sample solution: Use a portion of solution under test. Analysis

Samples: *Standard solution* and *Sample solution*  
Calculate the percentages of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) dissolved.

Tolerances: NLT 75% (Q) of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

## IMPURITIES

### • LIMIT OF FREE SALICYLIC ACID

Use glassware throughout this procedure. Perform this procedure on the same day the powder is removed from the Capsules.

Diluent: Add 1 mL of phosphoric acid to each L of methanol.

Standard solution: 0.0012 mg/mL of USP Salicylic Acid RS in *Diluent*. Use this solution promptly.

Sample solution: Nominally 0.65 mg/mL of aspirin from the contents of Capsules in solution prepared as follows. Transfer a suitable portion of the contents of NLT 20 Capsules, equivalent to about 65 mg of aspirin, to an appropriate container. Add 100.0 mL of *Diluent*, and shake for 1 min. Promptly filter a portion of this solution, discarding the first 15 mL of the filtrate, and use the clear filtrate within 20 min after the addition of the *Diluent*. If the intensity of the *Sample solution* greatly exceeds that of the *Standard solution*, the solution may be suitably diluted with *Diluent*.

### Instrumental conditions

Mode: Fluorescence

Excitation wavelength: 305 nm

Emission wavelength: 444 nm

### Analysis

Samples: *Standard solution* and *Sample solution*

Allow the *Samples* to equilibrate for 2 min in the fluorimeter.

Calculate the percentage of salicylic acid in the portion of Capsules taken (F):

$$\text{Result} = (I_u/I_s) \times (C_s/C_u) \times 100$$

$I_u$  = fluorescence intensity readings from the *Sample solution*

$I_s$  = fluorescence intensity readings from the *Standard solution*

$C_s$  = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 2.5% of salicylic acid

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Aspirin RS

USP Butalbital RS

USP Caffeine RS

USP Salicylic Acid RS

## Butalbital, Aspirin, and Caffeine Tablets

## DEFINITION

Butalbital, Aspirin, and Caffeine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ).

## IDENTIFICATION

• **A.** The retention times of the butalbital, aspirin, and caffeine peaks of the *Sample solution* correspond to those of the butalbital, aspirin, and caffeine peaks of the *Standard solution*, as obtained in the Assay.

## ASSAY

### • PROCEDURE

Buffer: 1.36 g/L of monobasic potassium phosphate in water

Mobile phase: Methanol and *Buffer* (45:55) initially adjusted with phosphoric acid to a pH of 3.9. If the retention time of the salicylic acid peak differs from that of the aspirin peak, adjust the pH of the *Mobile phase* with 0.2 N potassium hydroxide or 1 M phosphoric acid so that the salicylic acid peak has the same retention time as that of the aspirin peak. [NOTE—The retention time of the salicylic acid peak decreases about 0.3 min for each 0.1 pH increase. The retention time of the aspirin peak is essentially unaffected by such pH adjustments.]

Diluent: Methanol and *Buffer* (45:55) adjusted with phosphoric acid to a pH of  $2.5 \pm 0.05$ .

Salicylic acid solution: 0.1 mg/mL of salicylic acid in *Diluent*. Pass this solution through a suitable filter of 0.5- $\mu$ m or finer pore size.

Standard stock solution: 1.6 mg/mL of USP Aspirin RS in *Diluent*. Sonication and shaking may be used to aid in dissolution. Use this solution within 24 h.

Standard solution: USP Reference Standards in *Standard stock solution* as listed below. Sonication and shaking the solution may be used to promote dissolution. Use this solution within 24 h.

Butalbital: 1.6/ mg/mL of USP Butalbital RS, where  $I$  is the ratio of the labeled amount, in mg, of butalbital relative to the labeled amount of aspirin, in mg/Tablet

Caffeine: 1.6/ mg/mL of USP Caffeine RS, where  $I'$  is the ratio of the respective labeled amount, in mg, of caffeine relative to the labeled amount of aspirin in mg/Tablet

Sample solution: Nominally 1.6 mg/mL of aspirin from a suitable amount of powdered Tablets in solution prepared as follows. Finely powder NLT 20 Tablets, and transfer a portion of this fine powder to an appropriate volumetric flask. Dilute with *Diluent* to volume, and sonicate for 30 min. Pass a portion of this solution through a suitable filter of 0.5- $\mu$ m or finer pore size, and use the filtrate within 24 h.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detectors****Butalbital:** UV 210 nm**Aspirin and caffeine:** UV at the wavelength of the isosbestic point of aspirin and salicylic acid at about 277 nm**Column:** 3.9-mm × 30-cm; packing L1**Column temperature:** 35 ± 1°**Flow rate:** 1 mL/min**Injection volume:** 10 µL**System suitability****Samples:** *Salicylic acid solution* and *Standard solution*

[NOTE—The relative retention times for caffeine, aspirin, salicylic acid, and butalbital are about 0.45, 0.6, 0.6, and 1.0, respectively.]

**Suitability requirements****Resolution:** NLT 2.0 between caffeine and aspirin, *Standard solution***Column efficiency:** NLT 2000 theoretical plates from butalbital, *Standard solution***Relative standard deviation:** NMT 2.0% each for caffeine, aspirin, and butalbital responses, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ) and caffeine ( $C_8H_{10}N_4O_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 $r_u$  = peak response of butalbital or caffeine from the *Sample solution* $r_s$  = peak response of butalbital or caffeine from the *Standard solution* $C_s$  = concentration of USP Butalbital RS or USP Caffeine RS in the *Standard solution* (mg/mL) $C_u$  = nominal concentration of butalbital or caffeine in the *Sample solution* (mg/mL)Determine the amount, in mg, of aspirin and salicylic acid in the portion of Tablets taken ( $W$ ):

$$\text{Result} = (r_u/r_s) \times C_s \times V$$

 $r_u$  = peak response of aspirin and salicylic acid from the *Sample solution* $r_s$  = peak response of aspirin and salicylic acid from the *Standard solution* $C_s$  = concentration of USP Aspirin RS in the *Standard solution* (mg/mL) $V$  = volume of the *Sample solution* (mL)Calculate the percentage of the labeled amount of aspirin ( $C_9H_8O_4$ ) in the portion of Tablets taken:

$$\text{Result} = \{W - [(F/100) \times W]\} / (C_u \times V) \times 100$$

 $W$  = amount of aspirin and salicylic acid in the portion of Tablets taken to prepare the *Sample solution* (mg) $F$  = percentage of salicylic acid obtained in the *Limit of Free Salicylic Acid* procedure (%) $C_u$  = nominal concentration of aspirin in the *Sample solution* (mg/mL) $V$  = volume of the *Sample solution* (mL)**Acceptance criteria:** 90.0%–110.0% each of butalbital, aspirin, and caffeine**PERFORMANCE TESTS****• DISSOLUTION (711)****Medium:** Water; 900 mL**Apparatus 1:** 100 rpm**Time:** 60 min**Buffer, Mobile phase, Diluent, Salicylic acid solution, Standard solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.**Sample solution:** Use a portion of solution under test. **Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentages of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) dissolved.**Tolerances:** NLT 80% (Q) of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) is dissolved.**• UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**IMPURITIES****• LIMIT OF FREE SALICYLIC ACID**

Use glassware throughout this procedure. Perform this procedure on the same day the Tablets are powdered.

**Diluent:** Add 1 mL of phosphoric acid to each L of methanol.**Standard solution:** 0.0012 mg/mL of USP Salicylic Acid RS in *Diluent*. Use this solution promptly.**Sample solution:** Nominally 0.65 mg/mL of aspirin from a suitable amount of powdered Tablets in solution prepared as follows. Finely powder NLT 20 Tablets, and transfer a suitable portion of fine powder, equivalent to 65 mg of aspirin, to an appropriate container. Add 100.0 mL of *Diluent*, and shake by mechanical means for 15 min. Filter a portion of this solution, discarding the first 15 mL of the filtrate, and use the clear filtrate within 20 min after the addition of the *Diluent*. If the intensity of the *Sample solution* greatly exceeds that of the *Standard solution*, the solution may be suitably diluted with *Diluent*.**Instrumental conditions****Mode:** Fluorescence**Excitation wavelength:** 305 nm**Emission wavelength:** 444 nm**Analysis****Samples:** *Standard solution* and *Sample solution*Allow the *Samples* to equilibrate for 2 min in the fluorimeter.Calculate the percentage of salicylic acid in the portion of Tablets taken ( $F$ ):

$$\text{Result} = (I_u/I_s) \times (C_s/C_u) \times 100$$

 $I_u$  = fluorescence intensity readings from the *Sample solution* $I_s$  = fluorescence intensity readings from the *Standard solution* $C_s$  = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL) $C_u$  = nominal concentration of aspirin in the *Sample solution* (mg/mL)



Acceptance criteria: NMT 3.0% of salicylic acid

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
  - USP Aspirin RS
  - USP Butalbital RS
  - USP Caffeine RS
  - USP Salicylic Acid RS

### Butalbital, Aspirin, Caffeine, and Codeine Phosphate Capsules

#### DEFINITION

Butalbital, Aspirin, Caffeine, and Codeine Phosphate Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), caffeine ( $C_8H_{10}N_4O_2$ ), and codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

#### IDENTIFICATION

- **A.** The retention times of the butalbital, aspirin, caffeine, and codeine peaks of the *Sample solution* correspond to those of the butalbital, aspirin, caffeine, and codeine peaks of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

**Buffer:** 1.36 g/L of monobasic potassium phosphate in water

**Mobile phase:** Methanol and *Buffer* (45:55) initially adjusted with phosphoric acid to a pH of 3.9. If the retention time of the salicylic acid peak differs from that of the aspirin peak, adjust the pH of the *Mobile phase* with 0.2 N potassium hydroxide or 1 M phosphoric acid so that the salicylic acid peak has the same retention time as that of the aspirin peak. [NOTE—The retention time of the salicylic acid peak decreases about 0.3 min for each 0.1 pH increase. The retention time of the aspirin peak is essentially unaffected by such pH adjustments.]

**Diluent:** Methanol and *Buffer* (45:55) adjusted with phosphoric acid to a pH of  $2.5 \pm 0.05$ .

**Salicylic acid solution:** 0.1 mg/mL of salicylic acid in *Diluent*. Pass this solution through a suitable filter of 0.5- $\mu$ m or finer pore size.

**Standard stock solution:** 1.6 mg/mL of USP Aspirin RS in *Diluent*. Sonication and shaking may be used to promote dissolution. Use this solution within 24 h.

**Standard solution:** USP Reference Standards in *Standard stock solution* as listed below. Sonication and shaking the solution may be used to promote dissolution. Use this solution within 24 h.

**Butalbital:** 1.6/*j* mg/mL of USP Butalbital RS, where *j* is the ratio of the labeled amount of butalbital relative to the labeled amount of aspirin in mg/Capsule

**Caffeine:** 1.6/*j'* mg/mL of USP Caffeine RS, where *j'* is the ratio of the labeled amount of caffeine relative to the labeled amount of aspirin in mg/Capsule

**Codeine phosphate:** 1.6/*j''* mg/mL of USP Codeine Phosphate RS, where *j''* is the ratio of the labeled amount of codeine phosphate relative to the labeled amount of aspirin in mg/Capsule

**Sample solution:** Nominally 1.6 mg/mL of aspirin from the contents of Capsules in solution prepared as follows. Transfer a suitable portion of the contents of NLT 20 Capsules to an appropriate volumetric flask. Dilute with *Diluent* to volume, and sonicate for 30 min. Pass a portion of this solution through a suitable filter of 0.5- $\mu$ m pore size, and use the filtrate within 24 h.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector**

**Butalbital and codeine:** UV 210 nm

**Caffeine and aspirin:** UV at the wavelength of the isosbestic point of aspirin and salicylic acid at about 277 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Column temperature:**  $35 \pm 1^\circ$

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Samples:** *Salicylic acid solution* and *Standard solution*

[NOTE—The relative retention times for codeine, caffeine, aspirin, salicylic acid, and butalbital are about 0.3, 0.45, 0.6, 0.6, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between caffeine and aspirin, *Standard solution*

**Column efficiency:** NLT 2000 theoretical plates from butalbital, *Standard solution*

**Relative standard deviation:** NMT 2.0% each for codeine, caffeine, aspirin, and butalbital responses, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ) and caffeine ( $C_8H_{10}N_4O_2$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response of butalbital or caffeine from the *Sample solution*

*r<sub>S</sub>* = peak response of butalbital or caffeine from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Butalbital RS or USP Caffeine RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of butalbital or caffeine in the *Sample solution* (mg/mL)

Determine the amount of aspirin and salicylic acid in the portion of Capsules taken (*W*):

$$\text{Result} = (r_U/r_S) \times C_S \times V$$

*r<sub>U</sub>* = peak response of aspirin and salicylic acid from the *Sample solution*

*r<sub>S</sub>* = peak response of aspirin and salicylic acid from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Aspirin RS in the *Standard solution* (mg/mL)

*V* = volume of the *Sample solution* (mL)

Calculate the percentage of the labeled amount of aspirin ( $C_9H_8O_4$ ) in the portion of Capsules taken:

$$\text{Result} = \{W - [(F/100) \times W]\} / (C_U \times V) \times 100$$

*W* = amount of aspirin and salicylic acid in the portion of Capsules taken to prepare the *Sample solution* (mg)

*F* = percentage of salicylic acid obtained in the *Limit of Free Salicylic Acid* procedure (%)

*C<sub>U</sub>* = nominal concentration of aspirin in the *Sample solution* (mg/mL)

*V* = volume of the *Sample solution* (mL)

Calculate the percentage of the labeled amount of codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

*r<sub>U</sub>* = peak response of codeine from the *Sample solution*

*r<sub>S</sub>* = peak response of codeine from the *Standard solution*



- $C_S$  = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of codeine phosphate in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of codeine phosphate hemihydrate, 406.37  
 $M_{r2}$  = molecular weight of codeine phosphate anhydrous, 397.37  
**Acceptance criteria:** 90.0%–110.0% each of butalbital, aspirin, caffeine, and codeine phosphate

## PERFORMANCE TESTS

### • DISSOLUTION (711)

**Medium:** Water; 1000 mL

**Apparatus 2:** 50 rpm

**Time:** 60 min

**Buffer, Mobile phase, and Diluent:** Prepare as directed in the *Assay*.

**Salicylic acid solution:** 0.01 mg/mL of salicylic acid in *Diluent*. Pass this solution through a suitable filter of 0.5- $\mu$ m or finer pore size.

**Standard stock solution:** 0.16 mg/mL of USP Aspirin RS in a mixture of *Diluent* and *Medium* (50:50). Use this solution within 24 h.

**Standard solution:** USP Reference Standards in *Standard stock solution* as listed below. Sonication and shaking the solution may be used to promote dissolution. Pass a portion of the resulting solution through a suitable filter of 0.5- $\mu$ m or finer pore size. Use this solution within 24 h.

**Butalbital:** 0.16/ $j$  mg/mL of USP Butalbital RS, where  $j$  is the ratio of the labeled amount of butalbital relative to the labeled amount of aspirin in mg/Capsule

**Caffeine:** 0.16/ $j'$  mg/mL of USP Caffeine RS, where  $j'$  is the ratio of the labeled amount of caffeine relative to the labeled amount of aspirin in mg/Capsule

**Codeine phosphate:** 0.16/ $j''$  mg/mL of USP Codeine Phosphate RS, where  $j''$  is the ratio of the labeled amount of codeine phosphate relative to the labeled amount of aspirin in mg/Capsule

**Sample stock solution:** Pass 20 mL of the solution under test through a suitable filter of 0.5- $\mu$ m or finer pore size, discarding the first 2 mL of the filtrate.

**Sample solution:** A portion of the *Sample stock solution* diluted with an equal volume *Diluent*

**Chromatographic system and System suitability:** Proceed as directed in the *Assay*, except use an *Injection volume* of 100  $\mu$ L.

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentages of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), and caffeine ( $C_8H_{10}N_4O_2$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times 100$$

$r_U$  = peak response of the butalbital, aspirin, or caffeine from the *Sample solution*

$r_S$  = peak response of the butalbital, aspirin, or caffeine from the *Standard solution*

$C_S$  = concentration of USP Butalbital RS, USP Aspirin RS, or USP Caffeine RS in the *Standard solution* (mg/mL)

$V$  = volume of the *Medium*, 1000 mL

Calculate the percentage of the labeled amount of codeine phosphate ( $C_{18}H_{21}NO_3 \cdot \frac{1}{2}H_2O$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of codeine from the *Sample solution*

$r_S$  = peak response of codeine from the *Standard solution*

$C_S$  = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)

$V$  = volume of the *Medium*, 1000 mL

$M_{r1}$  = molecular weight of codeine phosphate hemihydrate, 406.37

$M_{r2}$  = molecular weight of codeine phosphate anhydrous, 397.37

**Tolerances:** NLT 75% (Q) of the labeled amounts of butalbital ( $C_{11}H_{16}N_2O_3$ ), aspirin ( $C_9H_8O_4$ ), caffeine ( $C_8H_{10}N_4O_2$ ), and codeine phosphate ( $C_{18}H_{21}NO_3 \cdot \frac{1}{2}H_2O$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### • LIMIT OF FREE SALICYLIC ACID

Use glassware throughout this procedure. Perform this procedure on the same day the powder is removed from the Capsules.

**Diluent:** To each L of methanol add 1 mL of phosphoric acid.

**Standard solution:** 0.0012 mg/mL of USP Salicylic Acid RS in *Diluent*. Use this solution promptly.

**Sample solution:** Nominally 0.65 mg/mL of aspirin from the contents of Capsules in solution prepared as follows. Transfer a suitable portion of the contents of NLT 20 Capsules, equivalent to about 65 mg of aspirin, to an appropriate container. Add 100.0 mL of *Diluent*, and shake for 1 min. Promptly filter a portion of this solution, discarding the first 15 mL of the filtrate, and use the clear filtrate within 20 min after the addition of the *Diluent*. If the intensity of the *Sample solution* greatly exceeds that of the *Standard solution*, the solution may be suitably diluted with *Diluent*.

### Instrumental conditions

**Mode:** Fluorescence

**Excitation wavelength:** 305 nm

**Emission wavelength:** 444 nm

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Allow the *Samples* to equilibrate for 2 min in the fluorimeter.

Calculate the percentage of salicylic acid in the portion of Capsules taken ( $F$ ):

$$\text{Result} = (I_U/I_S) \times (C_S/C_U) \times 100$$

$I_U$  = fluorescence intensity readings from the *Sample solution*

$I_S$  = fluorescence intensity readings from the *Standard solution*

$C_S$  = concentration of USP Salicylic Acid RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of aspirin in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 3.0% of salicylic acid

## ADDITIONAL REQUIREMENTS

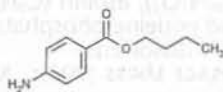
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.



• **USP REFERENCE STANDARDS** (11)

USP Aspirin RS  
USP Butalbital RS  
USP Caffeine RS  
USP Codeine Phosphate RS  
USP Salicylic Acid RS

## Butamben



$C_{11}H_{15}NO_2$  193.24  
Benzoic acid, 4-amino-, butyl ester.  
Butyl *p*-aminobenzoate [94-25-7].

» Butamben, dried over phosphorus pentoxide for 3 hours, contains not less than 98.0 percent and not more than 101.0 percent of  $C_{11}H_{15}NO_2$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Butamben RS

**Completeness and color of solution**—One g dissolves completely in 30 mL of alcohol and in 30 mL of ether, and the solutions are colorless.

**Identification, Infrared Absorption** (197K).

**Melting range, Class I** (741): between 57° and 59°.

**Reaction**—Dissolve 1 g in 10 mL of neutralized alcohol: a clear solution results. Dilute this solution with 10 mL of water, and add 2 drops of phenolphthalein TS and 1 drop of 0.1 N sodium hydroxide: a red color is produced.

**Loss on drying** (731)—Dry it over phosphorus pentoxide for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

**Chloride**—To a solution of 200 mg in 10 mL of alcohol add 1 mL of 2 N nitric acid and a few drops of silver nitrate TS: no opalescence is produced.

### Delete the following:

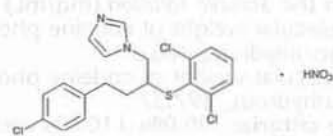
• **Heavy metals, Method I** (231)—Dissolve 2 g in 2 mL of 1 N acetic acid and sufficient alcohol to make 25 mL: the limit is 0.001%. • (Official 1-Jan-2018)

### Assay—

**Ferrocphen indicator solution**—Dissolve, without warming, 0.5 g of ferrocphen in 50 mL of sulfuric acid.

**Procedure**—Dissolve about 400 mg of Butamben, previously dried and accurately weighed, in a mixture of 100 mL of water and 20 mL of hydrochloric acid. Add 1 mL of *Ferrocphen indicator solution*. Cool the solution in an ice bath to about 10°, and titrate with 0.1 M sodium nitrite VS to a violet endpoint that is stable for not less than three minutes. Perform a blank determination, and make any necessary correction. Each mL of 0.1 M sodium nitrite is equivalent to 19.32 mg of  $C_{11}H_{15}NO_2$ .

## Butoconazole Nitrate



$C_{19}H_{17}Cl_3N_2S \cdot HNO_3$  474.79  
1*H*-Imidazole, 1-[4-(4-chlorophenyl)-2-[(2,6-dichlorophenyl)thio]butyl-, mononitrate, (±); (±)-1-[4-(*p*-Chlorophenyl)-2-[(2,6-dichlorophenyl)thio]butyl]imidazole mononitrate [64872-77-1].

### DEFINITION

Butoconazole Nitrate contains NLT 98.0% and NMT 102.0% of butoconazole nitrate ( $C_{19}H_{17}Cl_3N_2S \cdot HNO_3$ ), calculated on the dried basis.

### IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

### ASSAY

• **PROCEDURE**

**Buffer:** 2.18 g/L of monobasic potassium phosphate

and 4.18 g/L of dibasic potassium phosphate in water

**Mobile phase:** Methanol and *Buffer* (3:1)

**Standard solution:** 0.2 mg/mL of USP Butoconazole Nitrate RS in *Mobile phase*

**Sample solution:** 0.2 mg/mL of Butoconazole Nitrate in *Mobile phase*. Filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 229 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Column temperature:** 40°

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 2800 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 1.5%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of butoconazole nitrate

( $C_{19}H_{17}Cl_3N_2S \cdot HNO_3$ ) in the portion of Butoconazole Nitrate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Butoconazole Nitrate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Butoconazole Nitrate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **ORDINARY IMPURITIES** (466)

**Standard solutions:** USP Butoconazole Nitrate RS in a mixture of methylene chloride and methanol (2:1)

**Test solution:** Butoconazole Nitrate in a mixture of methylene chloride and methanol (2:1)

**Eluant:** Chloroform, tetrahydrofuran, cyclohexane, and ammonium hydroxide (18:18:13:1)



Visualization: 22  
Acceptance criteria: Meets the requirements

# SPECIFIC TESTS

## • LOSS ON DRYING (731)

Analysis: Dry under vacuum at 60° for 3 h.  
Acceptance criteria: NMT 1.0%

# ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Butoconazole Nitrate RS

## Butoconazole Nitrate Vaginal Cream

### DEFINITION

Butoconazole Nitrate Vaginal Cream contains Butoconazole Nitrate in a suitable cream base. It contains NLT 90.0% and NMT 110.0% of the labeled amount of butoconazole nitrate ( $C_{19}H_{17}Cl_3N_2S \cdot HNO_3$ ).

### IDENTIFICATION

#### • A.

**Sample solution:** Prepare a mixture of the *Standard solution* and the *Sample solution* (1:1), as directed in the *Assay*.

**Analysis:** Chromatograph the *Sample solution*, as directed in the *Assay*.

**Acceptance criteria:** The chromatogram exhibits two main peaks that correspond to butoconazole nitrate and the internal standard.

### ASSAY

#### • PROCEDURE

**Buffer:** 1.4 g of potassium acetate in 980 mL of water. Adjust with about 2 mL of glacial acetic acid to a pH of  $4.3 \pm 0.1$ , dilute with water to 1000 mL. Adjust the buffer molarity (0.018–0.072 M) as necessary to obtain suitable chromatographic performance. Increased retention time may be achieved by a decrease in the buffer molarity.

**Diluent:** Methanol and *Buffer* (60:40)

**Mobile phase:** Methanol and *Buffer* (65:35)

**Internal standard solution:** 1.6 mg/mL of 1-benzylimidazole in methanol

**Standard stock solution:** 0.4 mg/mL of USP Butoconazole Nitrate RS in methanol

**Standard solution:** Transfer 2.0 mL of the *Standard stock solution* and 3.0 mL of the *Internal standard solution* to a 50-mL flask, and add 35.0 mL of *Diluent*.

**Sample stock solution:** Nominally 0.4 mg/mL of butoconazole nitrate in methanol from Vaginal Cream prepared as follows. Add 200 mL of methanol to a 250-mL volumetric flask. Transfer to the flask a weighed quantity of Vaginal Cream equivalent to about 100 mg of butoconazole nitrate. Sonicate to dissolve, and cool to room temperature. Dilute with methanol to volume.

**Sample solution:** Transfer 2.0 mL of the *Sample stock solution* and 3.0 mL of the *Internal standard solution* to a 50-mL flask, and add 35.0 mL of *Diluent*. Allow the precipitated excipients that form to rise to the top of the solution, remove them by aspiration, and discard. Centrifuge or filter the remaining solution.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 225 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L9 that has been converted to the potassium form by the use of 0.555 M potassium acetate solution

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for butoconazole nitrate and 1-benzylimidazole are 0.6 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 4.0 between the analyte and internal standard peaks

**Column efficiency:** NLT 1100 theoretical plates for the analyte peak

**Tailing factor:** NMT 2.1 for the analyte peak

**Relative standard deviation:** NMT 1.5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of butoconazole nitrate ( $C_{19}H_{17}Cl_3N_2S \cdot HNO_3$ ) in the portion of Vaginal Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of butoconazole nitrate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of butoconazole nitrate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Butoconazole Nitrate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of butoconazole nitrate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount

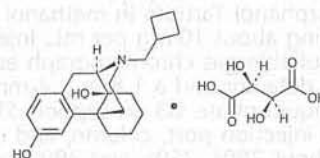
### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers. Avoid excessive heat, and avoid freezing.
- **USP REFERENCE STANDARDS (11)**  
USP Butoconazole Nitrate RS

## Butorphanol Tartrate



$C_{21}H_{29}NO_2 \cdot C_4H_6O_6$  477.55

Morphinan-3,14-diol, 17-(cyclobutylmethyl)-, (-)-, [S-(R\*, R\*)]-2,3-dihydroxybutanedioate (1:1) (salt).

(-)-17-(Cyclobutylmethyl)morphinan-3,14-diol D-(-)-tartrate (1:1) (salt) [58786-99-5].

» Butorphanol Tartrate contains not less than 98.0 percent and not more than 102.0 percent of  $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

#### USP Reference standards (11)—

USP Butorphanol Tartrate RS



**Identification—**

A: *Infrared Absorption* (197K).

B: The  $R_f$  value of the principal spot in the chromatogram of the *Test preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained for test A in the *Chromatographic purity* test.

**Specific rotation** (781S): between  $-60^\circ$  and  $-66^\circ$ .

*Test solution*: 4 mg per mL, in methanol.

**Water Determination, Method I** (921): not more than 2.0%.

**Residue on ignition** (281): not more than 0.1%.

**Delete the following:**

• **Heavy metals, Method II** (231): 0.003%. • (Official 1-Jan-2018)

**Chromatographic purity—**

METHOD A (Thin-Layer Chromatography)—

*Standard solution*—Prepare a solution in methanol containing 1 mg of USP Butorphanol Tartrate RS per mL.

*Test solution*—Transfer 100 mg of Butorphanol Tartrate to a 10-mL volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix.

*Iodoplatinate spray reagent*—Prepare a 1 in 10 solution of chloroplatinic acid in water. To 0.5 mL of this solution add 33 mL of water and 1 g of potassium iodide to obtain the spray reagent. Prepare fresh daily.

*Procedure*—Apply 50  $\mu$ L of the *Test solution*, containing 500  $\mu$ g of butorphanol tartrate, and 5  $\mu$ L and 10  $\mu$ L of the *Standard solution*, containing 5  $\mu$ g and 10  $\mu$ g of USP Butorphanol Tartrate RS, respectively, about 2 cm apart to a line parallel to and about 2 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a developing chamber containing, and equilibrated with, a mixture of chloroform, methanol, benzene, and ammonium hydroxide (85:25:20:5). Develop the chromatogram until the solvent front has moved about 10 cm above the line of application. Remove the plate, mark the solvent front, and allow the solvent to evaporate. Spray the plate with *Iodoplatinate spray reagent*. Estimate the percentage of the impurities present in the *Test solution* by comparing the intensities of secondary spots, if present, with the intensities of the principal spots obtained from the chromatograms of the *Standard solution*. The sum of the impurities observed is not greater than 2.0%.

METHOD B (Gas Chromatography)—Dissolve a suitable quantity of Butorphanol Tartrate in methanol to obtain a solution containing about 10 mg per mL. Inject 1  $\mu$ L of this solution into a suitable gas chromatograph equipped with a flame-ionization detector and a 1.8-m  $\times$  4-mm glass column containing 3% liquid phase G3 on support S1AB. The temperatures of the injection port, column, and detector are maintained at about 280°, 250°, and 290°, respectively. The carrier gas is nitrogen. Record a 30-minute chromatogram. Preferably using an electronic integrator, determine the areas of all peaks in the chromatogram excluding the area of the solvent. In a suitable chromatogram, the retention time for the alpha isomer of butorphanol tartrate is 1.2 relative to 1.0 for butorphanol tartrate; and the retention time of butorphanol tartrate is not less than 15 minutes. Calculate the percentage of synthesis precursors in the test specimen by the formula:

$$100A_v / A_s$$

in which  $A_v$  is the sum of the areas of all minor peaks; and  $A_s$  is the sum of the areas of the major and minor peaks. The limit is 2.0%.

**Assay**—Dissolve about 500 mg of Butorphanol Tartrate, accurately weighed, in 75 mL of glacial acetic acid. Add crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform

a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 47.76 mg of  $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ .

**Butorphanol Tartrate Injection**

» Butorphanol Tartrate Injection is a sterile solution of Butorphanol Tartrate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ . It may contain a suitable preservative and a buffer.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards (11)—**

USP Butorphanol Tartrate RS  
USP Endotoxin RS

**Identification**—Apply 10- $\mu$ L portions of the Injection and a Standard solution of USP Butorphanol Tartrate RS having the same concentration about 2 cm apart to a line parallel to and about 2 cm from the bottom of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Place the plate in a developing chamber containing a mixture of chloroform, ethyl acetate, and methanol (40:10:9), and develop the chromatogram until the solvent front has moved about 10 cm above the line of application. Remove the plate, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution. Benzethonium chloride, if present, is observed as a streaked zone near the point of application. Visualize the butorphanol spots by lightly spraying the plate with a 1 in 250 solution of bromocresol purple in dehydrated alcohol: butorphanol appears as a blue spot against a light yellow background.

**pH** (791): between 3.0 and 5.5.

**Bacterial Endotoxins Test** (85)—It contains not more than 88.0 USP Endotoxin Units per mg of butorphanol tartrate.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay—**

*Mobile phase*—Prepare a mixture of 0.05 M ammonium acetate and acetonitrile (3:1) adjusted by the addition of glacial acetic acid to a pH of 4.1. The mixture is appropriately filtered and degassed.

*Internal standard solution*—Dissolve about 50 mg of propylparaben in 5.0 mL of methanol contained in a 250-mL volumetric flask. Add water to volume, and mix.

*Standard preparation*—Transfer about 50 mg of USP Butorphanol Tartrate RS, accurately weighed, to a 25-mL volumetric flask containing 1.0 mL of 1 N sulfuric acid. Swirl the flask to dissolve the powder completely, add water to volume, and mix. Pipet 5 mL of the resulting solution into a 50-mL volumetric flask containing 10.0 mL of *Internal standard solution*. Add water to volume, mix, and filter through a microporous filter, discarding the first 5 mL of the filtrate and collecting the remainder in a suitable container.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 10 mg of butorphanol tartrate, to a 50-mL volumetric flask. Add 10.0 mL of *Internal standard solution*, mix, add water to volume, and mix. Filter through a microporous filter, discarding the first 5 mL



of the filtrate and collecting the remainder in a suitable container.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column that contains packing L11. The flow rate is about 2 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 1.5%, and the capacity factor for butorphanol tartrate is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, adjusting the flow rate and other operating parameters, if necessary, until satisfactory chromatography and peak responses are obtained. Record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 1.7 for propylparaben and 1.0 for butorphanol tartrate. Calculate the quantity, in mg, of  $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$  in each mL of the Injection taken by the formula:

$$50(C/V)(R_U/R_S)$$

in which C is the concentration, in mg per mL, of USP Butorphanol Tartrate RS in the *Standard preparation*; V is the volume, in mL, of Injection taken; and  $R_U$  and  $R_S$  are the peak response ratios of the butorphanol tartrate peak and the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Butorphanol Tartrate Nasal Solution

(Title for this monograph—not to change until February 1, 2017)

(Prior to February 1, 2017, the current practice of labeling the article of commerce with the name Butorphanol Tartrate Nasal Solution may be continued. Use of the name Butorphanol Tartrate Nasal Spray will be permitted as of August 1, 2014, but the use of this name will not be mandatory until February 1, 2017. The 30-month extension will provide the time needed by manufacturers and users to make necessary changes.)

### DEFINITION

Butorphanol Tartrate Nasal Spray is an aqueous solution of butorphanol tartrate for administration as a metered spray to the nasal mucosa. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of butorphanol tartrate ( $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 1.0 mg/mL of USP Butorphanol Tartrate RS in methanol

**Sample solution:** Prepare a composite solution by pooling the contents of three containers of Nasal Spray into a suitable vessel. Transfer 1.0 mL of pooled sample to a 10-mL volumetric flask, and dilute with methanol to volume.

**Developing solvent system:** Chloroform, methanol, benzene, and ammonium hydroxide (17:5:4:1)

[**CAUTION**—Prepare in a hood while wearing appropriate safety gloves, lab coat, and protective eyewear.]

**Spray reagent:** Prepare a 1-in-10 solution of chloroplatinic acid in water. To 0.5 mL of this solution, add 33 mL of water and 1 g of potassium iodide. Prepare fresh daily.

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in the chapter, except spray the plate with *Spray reagent*.

**Acceptance criteria:** The typical  $R_f$  value is 0.7 for butorphanol tartrate.

### ASSAY

#### PROCEDURE

**Buffer:** 3.4 g/L of 0.025 M monobasic potassium phosphate. Filter.

**Mobile phase:** Acetonitrile, triethylamine, and Buffer (15:2:85). Mix thoroughly, and adjust with 85.0% phosphoric acid to a pH of  $3.0 \pm 0.1$ .

**Standard solution:** 0.2 mg/mL of USP Butorphanol Tartrate RS in *Mobile phase*. Mix, and filter, discarding the first 2 mL of the filtrate. The *Standard solution* is stable for at least 108 h.

**Sample solution:** Nominally 0.2 mg/mL of butorphanol tartrate in *Mobile phase* prepared as follows. Prepare a composite solution by pooling a minimum of four containers of Nasal Spray into a suitable glass vessel. Transfer the equivalent of 20 mg of butorphanol tartrate to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 2 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

#### Columns

**Analytical:** 4.6-mm × 15-cm; 5-µm packing L11

**Guard:** 4.6-mm × 1-cm; 5-µm packing L11

**Column temperature:** 30°

**Flow rate:** 2.0 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of butorphanol tartrate ( $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ ) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Butorphanol Tartrate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of butorphanol tartrate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### IMPURITIES

#### ORGANIC IMPURITIES

**Buffer:** Prepare as directed in the *Assay*.

**Mobile phase:** Acetonitrile, triethylamine, and Buffer (15:5.1:85). Mix thoroughly, and adjust with 85.0% phosphoric acid to a pH of  $3.0 \pm 0.1$ .

**Standard solution:** 0.005 mg/mL of USP Butorphanol Tartrate RS

**Sensitivity solution:** Transfer 2.5 mL of the *Standard solution* to a 50-mL volumetric flask, and dilute with water to volume. Do not filter.

**Sample solution:** Nominally 1 mg/mL of butorphanol tartrate in water prepared as follows. Prepare a composite solution by pooling a minimum of four containers of Nasal Spray into a suitable glass vessel. Transfer the equivalent of 50 mg of butorphanol tartrate to a 50-mL volumetric flask. Dilute with water to volume. Do not filter.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Columns

Analytical: 4.6-mm × 25-cm; 5-μm packing L11

Guard: 4.6-mm × 1-cm; 5-μm packing L11

Column temperature: 40°

Flow rate: 2.0 mL/min

Injection volume: 60 μL

Run time: 40 min

**System suitability**Samples: *Standard solution* and *Sensitivity solution***Suitability requirements**Relative standard deviation: NMT 10.0%, *Standard solution*Sensitivity: The peak height for butorphanol tartrate is greater than or equal to three times the baseline noise, *Sensitivity solution***Analysis**Samples: *Standard solution* and *Sample solution*Record the chromatograms, and measure the responses for the butorphanol tartrate peak in the *Standard solution*, and for all known and unknown related compounds in the *Sample solution*.Calculate the percentage of each related compound (see *Table 1*) and each unknown impurity in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each known or unknown related compound from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Butorphanol Tartrate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of butorphanol tartrate in the *Sample solution* (mg/mL)Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
3,14-Dihydroxymorphinan	0.3	0.3
Δ6-Butorphanol	0.7	0.5
Butorphanol tartrate	1.0	—
Unknown impurity	—	0.3
Total impurities	—	1.0

**SPECIFIC TESTS**

- MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed  $10^3$  cfu/g or mL, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g or mL. It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

- PH** (791): 4.0–6.0

- OSMOLALITY AND OSMOLARITY** (785): 252–292 mOsmol/kg

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature. Store at 25°; excursions permitted between 15° and 30°.

- USP REFERENCE STANDARDS** (11)

USP Butorphanol Tartrate RS

**Butorphanol Tartrate Nasal Spray**

(Title for this monograph—not to change until February 1, 2017)

(Prior to February 1, 2017, the current practice of labeling the article of commerce with the name Butorphanol Tartrate Nasal Solution may be continued. Use of the name Butorphanol Tartrate Nasal Spray will be permitted as of August 1, 2014, but the use of this name will not be mandatory until February 1, 2017. The 30-month extension will provide the time needed by manufacturers and users to make necessary changes.)

**DEFINITION**

Butorphanol Tartrate Nasal Spray is an aqueous solution of butorphanol tartrate for administration as a metered spray to the nasal mucosa. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of butorphanol tartrate ( $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ ).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Standard solution:** 1.0 mg/mL of USP Butorphanol Tartrate RS in methanol

**Sample solution:** Prepare a composite solution by pooling the contents of three containers of Nasal Spray into a suitable vessel. Transfer 1.0 mL of pooled sample to a 10-mL volumetric flask, and dilute with methanol to volume.

**Developing solvent system:** Chloroform, methanol, benzene, and ammonium hydroxide (17:5:4:1)

[**CAUTION**—Prepare in a hood while wearing appropriate safety gloves, lab coat, and protective eyewear.]

**Spray reagent:** Prepare a 1-in-10 solution of chloroplatinic acid in water. To 0.5 mL of this solution, add 33 mL of water and 1 g of potassium iodide. Prepare fresh daily.

**Analysis**

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter, except spray the plate with *Spray reagent*.

Acceptance criteria: The typical  $R_f$  value is 0.7 for butorphanol tartrate.

**ASSAY**

- PROCEDURE**

**Buffer:** 3.4 g/L of 0.025 M monobasic potassium phosphate. Filter.

**Mobile phase:** Acetonitrile, triethylamine, and *Buffer* (15:2:85). Mix thoroughly, and adjust with 85.0% phosphoric acid to a pH of  $3.0 \pm 0.1$ .

**Standard solution:** 0.2 mg/mL of USP Butorphanol Tartrate RS in *Mobile phase*. Mix, and filter, discarding the first 2 mL of the filtrate. The *Standard solution* is stable for at least 108 h.

**Sample solution:** Nominally 0.2 mg/mL of butorphanol tartrate in *Mobile phase* prepared as follows. Prepare a composite solution by pooling a minimum of four containers of Nasal Spray into a suitable glass vessel. Transfer the equivalent of 20 mg of butorphanol tartrate to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 2 mL of the filtrate.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 280 nm**Columns****Analytical:** 4.6-mm × 15-cm; 5-μm packing L11**Guard:** 4.6-mm × 1-cm; 5-μm packing L11**Column temperature:** 30°**Flow rate:** 2.0 mL/min**Injection volume:** 20 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of butorphanol tartrate ( $C_{21}H_{29}NO_2 \cdot C_4H_6O_6$ ) in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Butorphanol Tartrate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of butorphanol tartrate in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**IMPURITIES****• ORGANIC IMPURITIES****Buffer:** Prepare as directed in the *Assay*.**Mobile phase:** Acetonitrile, triethylamine, and *Buffer* (15: 5.1: 85). Mix thoroughly, and adjust with 85.0% phosphoric acid to a pH of 3.0 ± 0.1.**Standard solution:** 0.005 mg/mL of USP Butorphanol Tartrate RS**Sensitivity solution:** Transfer 2.5 mL of the *Standard solution* to a 50-mL volumetric flask, and dilute with water to volume. Do not filter.**Sample solution:** Nominally 1 mg/mL of butorphanol tartrate in water prepared as follows. Prepare a composite solution by pooling a minimum of four containers of Nasal Spray into a suitable glass vessel. Transfer the equivalent of 50 mg of butorphanol tartrate to a 50-mL volumetric flask. Dilute with water to volume. Do not filter.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 280 nm**Columns****Analytical:** 4.6-mm × 25-cm; 5-μm packing L11**Guard:** 4.6-mm × 1-cm; 5-μm packing L11**Column temperature:** 40°**Flow rate:** 2.0 mL/min**Injection volume:** 60 μL**Run time:** 40 min**System suitability****Samples:** *Standard solution* and *Sensitivity solution***Suitability requirements****Relative standard deviation:** NMT 10.0%, *Standard solution***Sensitivity:** The peak height for butorphanol tartrate is greater than or equal to three times the baseline noise, *Sensitivity solution***Analysis****Samples:** *Standard solution* and *Sample solution*Record the chromatograms, and measure the responses for the butorphanol tartrate peak in the *Standard solution*, and for all known and unknown related compounds in the *Sample solution*.Calculate the percentage of each related compound (see *Table 1*) and each unknown impurity in the portion of Nasal Spray taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each known or unknown related compound from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Butorphanol Tartrate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of butorphanol tartrate in the *Sample solution* (mg/mL)**Acceptance criteria:** See *Table 1*.**Table 1**

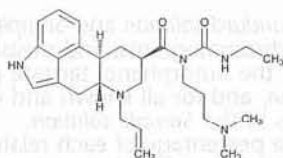
Name	Relative Retention Time	Acceptance Criteria, NMT (%)
3,14-Dihydroxymorphinan	0.3	0.3
Δ6-Butorphanol	0.7	0.5
Butorphanol tartrate	1.0	—
Unknown impurity	—	0.3
Total impurities	—	1.0

**SPECIFIC TESTS****• MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed  $10^3$  cfu/g or mL, and the total combined molds and yeasts count does not exceed  $10^2$  cfu/g or mL. It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.**• pH (791):** 4.0–6.0**• OSMOLALITY AND OSMOLARITY (785):** 252–292 mOsmol/kg**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature. Store at 25°; excursions permitted between 15° and 30°.**• USP REFERENCE STANDARDS (11)**

USP Butorphanol Tartrate RS



## Cabergoline



$C_{26}H_{37}N_5O_2$  451.60  
Ergoline-8 $\beta$ -carboxamide, *N*-[3-(dimethylamino)propyl]-*N*-[(ethylamino)carbonyl]-6-(2-propenyl)-; 1-[(6-Allylergolin-8 $\beta$ -yl)carbonyl]-1-[3-(dimethylamino)propyl]-3-ethylurea [81409-90-7].

### DEFINITION

Cabergoline contains NLT 98.0% and NMT 102.0% of cabergoline ( $C_{26}H_{37}N_5O_2$ ), calculated on the anhydrous basis for the crystalline form and on the anhydrous and solvent-free basis for the amorphous form.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

- B. Proceed as directed for *Procedure 1* or *Procedure 2*. The criteria for *Procedure 1* or *Procedure 2* must be met.

**Procedure 1: Crystallinity (695)**

**Acceptance criteria**

For the crystalline form: Meets the requirements

For the amorphous form: Does not meet the requirements

**Procedure 2: X-Ray Diffraction (941)**

**Acceptance criteria**

For the crystalline form: A diffraction pattern is present.

For the amorphous form: No diffraction pattern is present.

- C. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

Prepare solutions immediately before use, and protect from light.

**Buffer:** Dissolve 6.8 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 2.0, and dilute to 1 L. Add 0.2 mL of triethylamine to the resulting solution and mix.

**Mobile phase:** Acetonitrile and *Buffer* (4:21)

**Standard solution:** 0.25 mg/mL of USP Cabergoline RS in *Mobile phase*. Sonicate if needed.

**Sample solution:** 0.25 mg/mL of Cabergoline in *Mobile phase*. Sonicate if needed.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.0-mm  $\times$  25-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.3 mL/min

**Injection volume:** 100  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 1000 theoretical plates

**Relative standard deviation:** NMT 2.0% for five replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cabergoline ( $C_{26}H_{37}N_5O_2$ ) in the portion of Cabergoline taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Cabergoline RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Cabergoline in the *Sample solution* (mg/mL)

#### Acceptance criteria

For the crystalline form: 98.0%–102.0% on the anhydrous basis

For the amorphous form: 98.0%–102.0% on the anhydrous and solvent-free basis

### IMPURITIES

#### Delete the following:

- **HEAVY METALS, Method II (231):** 20 ppm (Official 1-Jan-2018)
- **RESIDUE ON IGNITION (281):** NMT 0.1%

#### • ORGANIC IMPURITIES

Prepare solutions immediately before use, and protect from light.

**Buffer and Mobile phase:** Proceed as directed in the *Assay*.

**System suitability solution:** To 10 mL of 0.1 M sodium hydroxide add 50 mg of Cabergoline and stir for about 15 min. To 1 mL of the suspension add 1 mL of 0.1 M hydrochloric acid, and dilute with *Mobile phase* to 10.0 mL. Sonicate until dissolution is complete. [NOTE—The main degradation product obtained is cabergoline related compound A.]

**Sample solution:** 0.25 mg/mL of Cabergoline in *Mobile phase*. Sonicate if needed.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.0-mm  $\times$  25-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.3 mL/min

**Injection volume:** 100  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—See *Table 1* for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 3.0 between cabergoline and cabergoline related compound A

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Cabergoline taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_T$  = sum of all the peak responses from the *Sample solution*

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cabergoline related compound D <sup>a</sup>	0.3	0.1
Cabergoline related compound B <sup>b</sup>	0.6	0.1

<sup>a</sup> (6 $\alpha$ R,9R,10 $\alpha$ R)-*N*-[3-(Dimethylamino)propyl]-7-(prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxamide.

<sup>b</sup> (6 $\alpha$ R,9R,10 $\alpha$ R)-*N*<sup>8</sup>-[3-(Dimethylamino)propyl]-*N*<sup>4</sup>-ethyl-7-(prop-2-enyl)-6a,7,8,9,10,10a-hexahydroindolo[4,3-*fg*]quinoline-4,9(6H)-dicarboxamide.

<sup>c</sup> (6 $\alpha$ R,9R,10 $\alpha$ R)-7-(Prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline-9-carboxylic acid.

<sup>d</sup> (6 $\alpha$ R,9R,10 $\alpha$ R)-*N*<sup>8</sup>-[3-(Dimethylamino)propyl]-*N*<sup>4</sup>-ethyl-*N*<sup>9</sup>-(ethyl-carbamoyl)-7-(prop-2-enyl)-6a,7,8,9,10,10a-hexahydroindolo[4,3-*fg*]quinoline-4,9(6H)-dicarboxamide.



Table 1 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cabergoline related compound A <sup>c</sup>	0.8	0.3
Cabergoline	1.0	—
Cabergoline related compound C <sup>d</sup>	2.9	0.3
Any other individual unidentified impurity	—	0.10
Total impurities	—	0.8

<sup>a</sup> (6aR,9R,10aR)-N-[3-(Dimethylamino)propyl]-7-(prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-fg]quinoline-9-carboxamide.

<sup>b</sup> (6aR,9R,10aR)-N<sup>o</sup>-[3-(Dimethylamino)propyl]-N<sup>o</sup>-ethyl-7-(prop-2-enyl)-6a,7,8,9,10,10a-hexahydroindolo[4,3-fg]quinoline-4,9(6H)-dicarboxamide.

<sup>c</sup> (6aR,9R,10aR)-7-(Prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-fg]quinoline-9-carboxylic acid.

<sup>d</sup> (6aR,9R,10aR)-N<sup>o</sup>-[3-(Dimethylamino)propyl]-N<sup>o</sup>-ethyl-N<sup>o</sup>-(ethyl-carbamoyl)-7-(prop-2-enyl)-6a,7,8,9,10,10a-hexahydroindolo[4,3-fg]quinoline-4,9(6H)-dicarboxamide.

### SPECIFIC TESTS

#### • OPTICAL ROTATION, Specific Rotation (781S)

Sample solution: 1 mg/mL in alcohol

##### Acceptance criteria

For the crystalline form:  $-77^{\circ}$  to  $-83^{\circ}$  on the anhydrous basis

For the amorphous form:  $-77^{\circ}$  to  $-83^{\circ}$  on the anhydrous and solvent-free basis

#### • WATER DETERMINATION, Method I (921)

##### Acceptance criteria

For the crystalline form: NMT 0.5%

For the amorphous form: NMT 1.5%

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. If it is labeled as amorphous, preserve under nitrogen in tight containers, store cold, and protect from light.

• **LABELING:** Where it is the amorphous form, the label so indicates.

#### • USP REFERENCE STANDARDS (11)

USP Cabergoline RS

## Cabergoline Tablets

### DEFINITION

Cabergoline Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cabergoline ( $C_{26}H_{37}N_5O_2$ ).

### IDENTIFICATION

• **A.** The retention time of the major peak in the *Sample solution* corresponds to the major peak in the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

Prepare solutions immediately before use, and protect from light.

**Buffer:** Transfer 6.8 g of monobasic potassium phosphate to a 1-L volumetric flask. Dissolve the contents in 900 mL of water. Adjust with phosphoric acid to a pH of 2.0. Dilute with water to volume, and add 0.2 mL of triethylamine.

**Mobile phase:** Acetonitrile and *Buffer* (16:84)

**Standard solution:** 0.25 mg/mL of USP Cabergoline RS in *Mobile phase*. Sonication may be used to aid in the dissolution of cabergoline.

**Sample solution:** Nominally 0.25 mg/mL of cabergoline from finely powdered Tablets in solution prepared as follows. Finely powder NLT 20 Tablets, and

transfer a suitable portion of this fine powder to an appropriate volumetric flask. Dilute with *Mobile phase* to volume, and sonicate until completely dissolved. The resulting solution may be passed through a PVDF-type filter with a pore size of 0.45  $\mu$ m before analysis.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.0-mm  $\times$  25-cm; 10- $\mu$ m packing L1

**Flow rate:** 1.3 mL/min

**Injection volume:** 100  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

##### Suitability requirements

**Column efficiency:** NLT 1000 theoretical plates

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cabergoline ( $C_{26}H_{37}N_5O_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cabergoline RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cabergoline in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid; 500 mL, degassed with helium

**Apparatus 2:** 50 rpm

**Time:** 15 min

**Buffer:** Transfer 6.8 g of monobasic potassium phosphate to a 1-L volumetric flask. Dissolve the contents in 900 mL of water. Adjust with phosphoric acid to a pH of 2.0. Dilute with water to volume, and add 0.2 mL of triethylamine.

**Mobile phase:** Acetonitrile and *Buffer* (16:84)

**Standard solution:** 1  $\mu$ g/mL of USP Cabergoline RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter, discarding the first few mL.

**Chromatographic system:** Proceed as directed in the *Assay*.

#### System suitability

**Sample:** *Standard solution*

##### Suitability requirements

**Column efficiency:** NLT 3000 theoretical plates

**Relative standard deviation:** NMT 2%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cabergoline ( $C_{26}H_{37}N_5O_2$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 500 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of cabergoline ( $C_{26}H_{37}N_5O_2$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements



**IMPURITIES****• ORGANIC IMPURITIES**

Prepare solutions immediately before use, and protect from light.

**Buffer:** Transfer 6.8 g of monobasic potassium phosphate to a 1-L volumetric flask. Dissolve the contents in 900 mL of water. Adjust with phosphoric acid to a pH of 2.0. Dilute with water to volume, and add 0.2 mL of triethylamine.

**Mobile phase:** Acetonitrile and *Buffer* (16:84)

**System suitability solution:** To 10 mL of 0.1 M sodium hydroxide, add 50 mg of cabergoline. Stir for 15 min. To 1 mL of the suspension add 1 mL of 0.1 M hydrochloric acid, and dilute with *Mobile phase* to 10 mL. Sonicate until dissolution is complete. The main degradation product obtained is cabergoline related compound A.

**Sample solution:** Nominally 0.25 mg/mL of cabergoline from finely powdered Tablets in solution prepared as follows. Finely powder NLT 20 Tablets, and transfer a suitable portion of this fine powder to an appropriate volumetric flask. Dilute with *Mobile phase* to volume, and sonicate until completely dissolved. The resulting solution may be passed through a PVDF-type filter with a pore size of 0.45 µm before analysis.

**Chromatographic system:** Proceed as directed in the *Assay*, except for the *Injection volume*.

**Injection volume**

**System suitability solution:** 20 µL

**Sample solution:** 100 µL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—See *Table 1* for relative retention times.]

**Suitability requirements**

**Resolution:** NLT 3.0 between cabergoline and cabergoline related compound A

**Analysis**

**Samples:** *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = response of each impurity from the *Sample solution*

$r_T$  = sum of responses of all impurities and cabergoline from the *Sample solution*

Calculate the percentage of total impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = sum of responses of all impurities from the *Sample solution*

$r_T$  = sum of responses of all impurities and cabergoline from the *Sample solution*

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cabergoline related compound A <sup>a</sup>	0.8	2.0
Cabergoline	1.0	—

<sup>a</sup> (6aR,9R,10aR)-7-(Prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-fg]quinoline-9-carboxylic acid.

<sup>b</sup> (6aR,9R,10aR)-7-Allyl-N-(3-(dimethylazirino)propyl)-N-(ethylcarbamoyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-fg]quinoline-9-carboxamide.

**Table 1 (Continued)**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cabergoline N-oxide <sup>b</sup>	1.4	1.0
Any unspecified degradation product	—	0.5
Total impurities	—	2.5

<sup>a</sup> (6aR,9R,10aR)-7-(Prop-2-enyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-fg]quinoline-9-carboxylic acid.

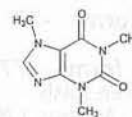
<sup>b</sup> (6aR,9R,10aR)-7-Allyl-N-(3-(dimethylazirino)propyl)-N-(ethylcarbamoyl)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-fg]quinoline-9-carboxamide.

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in light-resistant, tight containers, and store at controlled room temperature.

**• USP REFERENCE STANDARDS (11)**

USP Cabergoline RS

**Caffeine**

$C_8H_{10}N_4O_2 \cdot H_2O$  212.21

$C_8H_{10}N_4O_2$  194.19

1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-; 1,3,7-Trimethylxanthine [58-08-2].

Monohydrate [5743-12-4].

**DEFINITION**

Caffeine is anhydrous or contains one molecule of water of hydration. It contains NLT 98.5% and NMT 101.0% of  $C_8H_{10}N_4O_2$ , calculated on the anhydrous basis.

**IDENTIFICATION**

**• A. INFRARED ABSORPTION (197M)**

**• B.** The retention time of the caffeine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Buffer:** 0.82 g/L of anhydrous sodium acetate

**Mobile phase:** Acetonitrile, tetrahydrofuran, and *Buffer* (25:20:955). Adjust with glacial acetic acid to a pH of 4.5.

**System suitability solution:** 0.02 mg/mL of theophylline in *Mobile phase*. Shake, and sonicate if necessary, to dissolve.

**Standard solution:** Transfer 5.0 mg of USP Caffeine RS to a 25-mL volumetric flask. Add 5.0 mL of the *System suitability solution* and 10 mL of *Mobile phase*. Shake, and sonicate if necessary. Dilute with *Mobile phase* to volume, and filter.

**Sample solution:** 0.2 mg/mL of Caffeine in *Mobile phase*. [NOTE—Shake, and sonicate if necessary, to dissolve.]



**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 15-cm; packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

**System suitability**Sample: *Standard solution*

[NOTE—The relative retention times for theophylline and caffeine are 0.69 and 1.0, respectively.]

**Suitability requirements**

Resolution: NLT 6.0 between theophylline and caffeine

Tailing factor: NMT 2.0 for theophylline and caffeine peaks

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of caffeine (C<sub>8</sub>H<sub>10</sub>N<sub>4</sub>O<sub>2</sub>) in the portion of Caffeine taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 $r_u$  = peak response of caffeine from the *Sample solution* $r_s$  = peak response of caffeine from the *Standard solution* $C_s$  = concentration of USP Caffeine RS in the *Standard solution* (mg/mL) $C_u$  = concentration of Caffeine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the anhydrous basis

**IMPURITIES**

- **RESIDUE ON IGNITION** <281>: NMT 0.1%

**Delete the following:**

- **HEAVY METALS**, *Method II* <231>: NMT 10 ppm (Official 1, Jan-2018)

• **ORGANIC IMPURITIES**Mobile phase, *Standard solution*, *Sample solution*, *Chromatographic system*, and *System suitability*: Proceed as directed in the *Assay*.**Analysis**Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Caffeine taken:

$$\text{Result} = (r_u/r_T) \times 100$$

 $r_u$  = peak response for each impurity from the *Sample solution* $r_T$  = sum of the responses of all the peaks from the *Sample solution***Acceptance criteria**

Individual impurities: NMT 0.1%

Total impurities: NMT 0.1%

**SPECIFIC TESTS**

- **WATER DETERMINATION**, *Method III* <921>: Dry a sample at 80° for 4 h; the anhydrous form loses NMT 0.5% of its weight, and the hydrous form loses NMT 8.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve hydrous Caffeine in tight containers. Preserve anhydrous Caffeine in well-closed containers.
- **LABELING**: Label it to indicate whether it is anhydrous or hydrous.

• **USP REFERENCE STANDARDS** <11>

USP Caffeine RS

**Caffeine Citrate Injection****DEFINITION**Caffeine Citrate Injection is a sterile solution containing Caffeine and citric acid in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of caffeine citrate (C<sub>14</sub>H<sub>18</sub>N<sub>4</sub>O<sub>9</sub>). It contains no bacteriostat or other preservative.**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL**, *Citrate* <191>: Meets the requirements
- **C.**

**Solution A:** Transfer 4 g of potassium iodide to a 100-mL volumetric flask, and add 10 mL of water. Shake until the potassium iodide is dissolved. Add 2 g of iodine to the volumetric flask, and shake until dissolved. Dilute with water to volume.**Sample solution:** 5.0 mL of Injection**Analysis:** Transfer the *Sample solution* to a 25-mL centrifuge tube, and add 5 drops of *Solution A*. Add 0.5 mL of 2.0 M hydrochloric acid.**Acceptance criteria:** A brown precipitate that dissolves on neutralization with 0.5 mL of sodium hydroxide TS is produced.**ASSAY**• **PROCEDURE****Mobile phase:** Acetonitrile, tetrahydrofuran, and 0.01 M sodium acetate (5:4:191) adjusted with glacial acetic acid to a pH of 4.5**Theophylline stock solution:** 0.02 mg/mL of theophylline in water**Standard solution:** 0.2 mg/mL of USP Caffeine RS and 0.004 mg/mL of theophylline from *Theophylline stock solution* in water**Sample solution:** Nominally 0.4 mg/mL of caffeine citrate (equivalent to 0.2 mg/mL of caffeine) from Injection in water prepared as follows. Transfer a volume of Injection, equivalent to 50 mg of caffeine, to a 250-mL volumetric flask. Dilute with water to volume, pass through a polyvinylidene difluoride or equivalent membrane of 0.45-µm pore size, and use the filtrate.**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

**System suitability**Sample: *Standard solution*[NOTE—See *Table 1* for the relative retention times.]**Suitability requirements**

Resolution: NLT 6.0 between theophylline and caffeine

Tailing factor: NMT 2.0 each for theophylline and caffeine

Relative standard deviation: NMT 2.0% for caffeine

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the concentration ( $C_A$ ), in mg/mL, of caffeine citrate in the *Sample solution*:

$$\text{Result} = (r_u/r_s) \times C_s \times (M_{r1}/M_{r2})$$



- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)  
 $M_{r1}$  = molecular weight of caffeine citrate, 386.31  
 $M_{r2}$  = molecular weight of caffeine, 194.19  
 Calculate the percentage of the labeled amount of caffeine citrate ( $C_{14}H_{18}N_4O_9$ ) in the portion of Injection taken:

$$\text{Result} = (C_A/C_U) \times 100$$

- $C_A$  = concentration of caffeine citrate in the *Sample solution*  
 $C_U$  = nominal concentration of caffeine citrate in the *Sample solution*

Acceptance criteria: 90.0%–110.0%

## IMPURITIES

### • ORGANIC IMPURITIES

Mobile phase, Theophylline stock solution, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Sensitivity solution: 5 µg/mL of caffeine and 0.1 µg/mL of theophylline from the *Standard solution* in water

System suitability

Sample: *Sensitivity solution*

Suitability requirements

Sensitivity: The theophylline peak is discernible.

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_A) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

- $r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of caffeine from the *Standard solution*  
 $C_S$  = concentration of USP Caffeine RS in the *Standard solution* (mg/mL)  
 $C_A$  = concentration of caffeine citrate in the *Sample solution* as determined in the Assay  
 $M_{r1}$  = molecular weight of caffeine citrate, 386.31  
 $M_{r2}$  = molecular weight of caffeine, 194.19  
 $F$  = relative response factor (see Table 1)  
 Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Theobromine	0.4	1.13	0.10
Paraxanthine	0.6	0.909	0.10
Theophylline	0.7	1.10	0.10
Caffeine	1.0	—	—
Any individual impurity	—	1.0	0.10
Total impurities	—	—	0.1

## SPECIFIC TESTS

### • COLOR AND CLARITY

Analysis: Transfer a suitable portion of the Injection to a clear glass test tube, and visually examine the solution in a well-lighted area.

Acceptance criteria: The solution is colorless and free of haze, obvious turbidity, and precipitate.

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.25 USP Endotoxin Units/mg of caffeine
- **STERILITY TESTS** (71): It meets the requirements of the *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **pH** (791): 4.2–5.2
- **PARTICULATE MATTER IN INJECTIONS** (788): NMT 150 particles are equal to or greater than 10 µm, and NMT 25 particles are equal to or greater than 25 µm.
- **OTHER REQUIREMENTS**: It meets the requirements under *Injections and Implanted Drug Products* (1).

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in single-dose, tight containers of Type I glass, and store between 15°–30°.
- **USP REFERENCE STANDARDS** (11)  
 USP Caffeine RS  
 USP Endotoxin RS

## Caffeine Citrate Oral Solution

### DEFINITION

Caffeine Citrate Oral Solution is a sterile aqueous solution containing Caffeine and citric acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of caffeine citrate ( $C_{14}H_{18}N_4O_9$ ). It contains no bacteriostat or other preservative.

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B. IDENTIFICATION TESTS—GENERAL, Citrate** (191): Meets the requirements
- **C.**

**Solution A:** Transfer 4 g of potassium iodide to a 100-mL volumetric flask, and add 10 mL of water. Shake until the potassium iodide is dissolved. Add 2 g of iodine to the volumetric flask, and shake until dissolved. Dilute with water to volume.

**Sample solution:** 5.0 mL of Oral Solution

**Analysis:** Transfer the *Sample solution* to a 25-mL centrifuge tube, and add 5 drops of *Solution A*. Add 0.5 mL of 2.0 M hydrochloric acid.

**Acceptance criteria:** A brown precipitate that dissolves on neutralization with 0.5 mL of sodium hydroxide TS is produced.

## ASSAY

### • PROCEDURE

**Mobile phase:** Acetonitrile, tetrahydrofuran, and 0.01 M sodium acetate (5:4:191) adjusted with glacial acetic acid to a pH of 4.5

**Theophylline stock solution:** 0.02 mg/mL of theophylline in water

**Standard solution:** 0.2 mg/mL of USP Caffeine RS and 0.004 mg/mL of theophylline from *Theophylline stock solution* in water

**Sample solution:** Nominally 0.4 mg/mL of caffeine citrate (equivalent to 0.2 mg/mL of caffeine) from Oral Solution in water prepared as follows. Transfer a volume of Oral Solution, equivalent to 50 mg of caffeine, to a 250-mL volumetric flask. Dilute with water to volume, pass through a polyvinylidene difluoride or equivalent membrane of 0.45-µm pore size, and use the filtrate.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 275 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

**System suitability**Sample: *Standard solution*[NOTE—See *Table 1* for the relative retention times.]**Suitability requirements**

Resolution: NLT 6.0 between theophylline and caffeine

Tailing factor: NMT 2.0 each for the theophylline and caffeine

Relative standard deviation: NMT 2.0% for caffeine

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the concentration ( $C_A$ ), in mg/mL, of caffeine citrate in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2})$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Caffeine RS in the *Standard solution* (mg/mL) $M_{r1}$  = molecular weight of caffeine citrate, 386.31 $M_{r2}$  = molecular weight of caffeine, 194.19Calculate the percentage of the labeled amount of caffeine citrate ( $C_{14}H_{18}N_4O_9$ ) in the portion of Oral Solution taken:

$$\text{Result} = (C_A/C_U) \times 100$$

 $C_A$  = concentration of caffeine citrate in the *Sample solution* $C_U$  = nominal concentration of caffeine citrate in the *Sample solution*

Acceptance criteria: 90.0%–110.0%

**IMPURITIES****• ORGANIC IMPURITIES**Mobile phase, Theophylline stock solution, *Standard solution*, *Sample solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.Sensitivity solution: 5 μg/mL of caffeine and 0.1 μg/mL of theophylline from the *Standard solution* in water**System suitability**Sample: *Sensitivity solution***Suitability requirements**

Sensitivity: The theophylline peak is discernible.

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_A) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution* $r_S$  = peak response of caffeine from the *Standard solution* $C_S$  = concentration of USP Caffeine RS in the *Standard solution* (mg/mL) $C_A$  = concentration of caffeine citrate in the *Sample solution* as determined in the *Assay* $M_{r1}$  = molecular weight of caffeine citrate, 386.31 $M_{r2}$  = molecular weight of caffeine, 194.19 $F$  = relative response factor, (see *Table 1*)Acceptance criteria: See *Table 1*.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Theobromine	0.4	1.13	0.10
Paraxanthine	0.6	0.909	0.10
Theophylline	0.7	1.10	0.10
Caffeine	1.0	—	—
Any individual impurity	—	1.0	0.10
Total impurities	—	—	0.1

**SPECIFIC TESTS**• **STERILITY TESTS** (71): It meets the requirements of the *Test for Sterility of the Product to Be Examined, Membrane Filtration*.• **pH** (791): 4.2–5.2**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE**: Preserve in single-dose, tight containers, and store at a temperature between 15°–30°.• **USP REFERENCE STANDARDS** (11)  
USP Caffeine RS**Caffeine and Sodium Benzoate Injection****DEFINITION**Caffeine and Sodium Benzoate Injection is a sterile solution containing equal amounts of Caffeine and Sodium Benzoate in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amounts of anhydrous caffeine ( $C_8H_{10}N_4O_2$ ) and sodium benzoate ( $C_7H_5NaO_2$ ).**IDENTIFICATION****• A. INFRARED ABSORPTION** (197M)Sample: Use the residue from the *Assay for Caffeine*.

Acceptance criteria: Meets the requirements

**• B.**

Analysis: Dip the end of a platinum wire into a portion of Injection, and introduce it into a nonluminous flame.

Acceptance criteria: The flame is colored intensely yellow.

**• C.****Analysis**

Part 1: Add a few drops of ferric chloride TS to a 0.5-mL portion of Injection.

Part 2: Add 3 N hydrochloric acid to another portion of Injection.

Acceptance criteria: The criteria in *Part 1* and *Part 2* must both be met.

Part 1: A salmon-colored precipitate is formed.

Part 2: A white precipitate is formed.

**ASSAY****• CAFFEINE**

Sample solution: A volume of Injection equivalent to 250 mg each of caffeine and sodium benzoate

Analysis: Transfer the *Sample solution* with the aid of 5 mL of water to a small separator, add 1 drop of phenolphthalein TS, and add 0.1 N sodium hydroxide, dropwise, until a permanent pink color is just produced. Shake the mixture with three or more 20-mL portions of chloroform to effect complete extraction of the caffeine, passing each chloroform extract through a small filter previously moistened with chloroform into a tared dish. Retain the water layer for the *Assay for Sodium Benzoate*. Wash the stem of the separator, the filter, and the funnel with 10 mL of hot chloroform, adding the washings to the dish. Evaporate the combined chloro-



form solutions on a steam bath, adding 2 mL of alcohol just before the last trace of chloroform is expelled. Complete the evaporation of the solvent, dry the residue, consisting of caffeine ( $C_8H_{10}N_4O_2$ ), at 80° for 4 h. Cool, and weigh.

Acceptance criteria: 90.0%–110.0%

#### • SODIUM BENZOATE

**Sample solution:** Use the water layer obtained in the Assay for Caffeine.

#### Titrimetric system

**Mode:** Direct titration

**Titrant:** 0.1 N hydrochloric acid VS

**Endpoint detection:** Visual

**Analysis:** Add 75 mL of ether and 5 drops of methyl orange TS to the *Sample solution*. Titrate with *Titrant*, and shake vigorously until a permanent pink color is produced in the water layer. Each mL of 0.1 N hydrochloric acid is equivalent to 14.41 mg of sodium benzoate ( $C_7H_5NaO_2$ ).

Acceptance criteria: 90.0%–110.0%

#### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.7 USP Endotoxin Unit/mg of caffeine and sodium benzoate, based on the total, in mg, of the labeled amounts
- **PH (791):** 6.5–8.5
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products (1)*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS (11)**  
USP Caffeine RS  
USP Endotoxin RS

## Calamine

Iron oxide ( $Fe_2O_3$ ), mixture with zinc oxide;  
Calamine (pharmaceutical preparation) [8011-96-9].

#### DEFINITION

Calamine is Zinc Oxide with a small proportion of ferric oxide, and contains, after ignition, NLT 98.0% and NMT 100.5% of zinc oxide ( $ZnO$ ).

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Zinc (191)**  
**Sample:** 1 g  
**Analysis:** Treat the *Sample* with 10 mL of 3 N hydrochloric acid, and filter.  
**Acceptance criteria:** The filtrate meets the requirements.
- **B.**  
**Sample:** 1 g  
**Analysis:** Add 10 mL of 3 N hydrochloric acid to the *Sample*, heat to boil, and filter.  
**Acceptance criteria:** The filtrate assumes a reddish color upon the addition of ammonium thiocyanate TS.

#### ASSAY

##### • PROCEDURE

**Sample solution:** Digest 1.5 g of freshly ignited Calamine in 50.0 mL of 1 N sulfuric acid VS, applying gentle heat until no further solution occurs. Filter the mixture, and wash the residue on the filter with hot water until the last washing is neutral to litmus paper. To the combined filtrate and washings add 2.5 g of ammonium chloride. Cool, and add methyl orange TS.

#### Titrimetric system

**Mode:** Back titration

**Titrant:** 1 N sodium hydroxide VS

**Endpoint detection:** Visual

**Analysis:** Titrate the excess sulfuric acid in the *Sample solution* with *Titrant*. Each mL of 1 N sulfuric acid consumed is equivalent to 40.69 mg of zinc oxide ( $ZnO$ ).

**Acceptance criteria:** 98.0%–100.5% on the ignited basis

#### IMPURITIES

- **ARSENIC, Method I (211):** NMT 8 ppm

#### • CALCIUM

**Sample:** 1 g

**Analysis:** Digest the *Sample* in 25 mL of 3 N hydrochloric acid for 30 min, filter to remove the insoluble ferric oxide, and add 6 N ammonium hydroxide to the filtrate until the precipitate first formed is redissolved, then add 5 mL more of 6 N ammonium hydroxide. To 10 mL of this solution add 2 mL of ammonium oxalate TS.

**Acceptance criteria:** NMT a slight turbidity is produced.

#### • CALCIUM OR MAGNESIUM

**Analysis:** To another 10-mL portion of the solution prepared in the test for *Calcium*, add 2 mL of dibasic sodium phosphate TS.

**Acceptance criteria:** NMT a slight turbidity is produced.

#### • LEAD

**Sample:** 1 g

**Analysis:** Add 15 mL of water to the *Sample*, stir, then add 3 mL of glacial acetic acid, and warm on a steam bath until dissolved. Filter, and add 5 drops of potassium chromate TS.

**Acceptance criteria:** No turbidity is produced.

#### SPECIFIC TESTS

##### • ACID-INSOLUBLE SUBSTANCES

**Sample:** 2.0 g

**Analysis:** Dissolve the *Sample* in 50 mL of 3 N hydrochloric acid. If an insoluble residue remains, collect it on a tared filter, wash with water, and dry at 105° for 1 h. Cool, and weigh.

**Acceptance criteria:** NMT 40 mg (2.0%)

##### • ALKALINE SUBSTANCES

**Sample:** 1.0 g

**Analysis:** Digest the *Sample* with 20 mL of water on a steam bath for 15 min, filter, and add 2 drops of phenolphthalein TS.

**Acceptance criteria:** If a red color is produced, NMT 0.20 mL of 0.10 N sulfuric acid is required to discharge it.

##### • LOSS ON IGNITION (733)

**Sample:** 2 g

**Analysis:** Ignite the *Sample* at 500° to constant weight.

**Acceptance criteria:** NMT 2.0%

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## Calamine Topical Suspension

» Prepare Calamine Topical Suspension as follows.



Calamine .....	80 g
Zinc Oxide .....	80 g
Glycerin .....	20 mL
Bentonite Magma .....	250 mL
Calcium Hydroxide Topical Solution, a sufficient quantity to make	1000 mL

Dilute the Bentonite Magma with an equal volume of Calcium Hydroxide Topical Solution. Mix the powders intimately with the Glycerin and about 100 mL of the diluted magma, triturating until a smooth, uniform paste is formed. Gradually incorporate the remainder of the diluted magma. Finally add enough Calcium Hydroxide Topical Solution to make 1000 mL, and shake.

If a more viscous consistency in the Calamine Topical Suspension is desired, the quantity of Bentonite Magma may be increased to not more than 400 mL.

NOTE—Shake the Calamine Topical Suspension before dispensing.

**Packaging and storage**—Preserve in tight containers.

**Microbial enumeration tests (61) and Tests for specified microorganisms (62)**—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

### Phenolated Calamine Topical Suspension

» Prepare Phenolated Calamine Topical Suspension as follows:

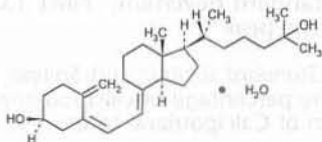
Liquefied Phenol .....	10 mL
Calamine Topical Suspension ....	990 mL
To make .....	1000 mL

Mix the ingredients.

NOTE—Shake Phenolated Calamine Topical Suspension before dispensing.

**Packaging and storage**—Preserve in tight containers.

### Calcifediol



$C_{27}H_{44}O_2 \cdot H_2O$  418.65

9,10-Secocholesta-5,7,10(19)-triene-3,25-diol monohydrate, (3 $\beta$ ,5Z,7E)-.

25-Hydroxycholecalciferol monohydrate [63283-36-3].

» Calcifediol contains not less than 97.0 percent and not more than 103.0 percent of  $C_{27}H_{44}O_2 \cdot H_2O$ .

**Packaging and storage**—Preserve in tight, light-resistant containers at controlled room temperature.

**USP Reference standards (11)**—

USP Calcifediol RS

**Identification, Infrared Absorption (197M).**

**Water Determination, Method Ia (921):** between 3.8% and 5.0%, determined on a 0.2-g specimen.

**Assay—**

**Internal standard solution**—Dissolve testosterone in ethyl acetate to obtain a solution having a concentration of about 0.10 mg per mL.

**Mobile phase**—Prepare a suitable degassed solution of about 6 volumes of heptane, 6 volumes of water-saturated heptane, 3 volumes of methylene chloride, and 5 volumes of ethyl acetate.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Calcifediol RS in *Internal standard solution*, and dilute quantitatively and stepwise with *Internal standard solution* to obtain a solution having a known concentration of about 20  $\mu$ g per mL.

**Assay preparation**—Transfer about 10 mg of Calcifediol, accurately weighed, to a 50-mL volumetric flask, dissolve in *Internal standard solution*, dilute with *Internal standard solution* to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with *Internal standard solution* to volume, and mix.

**Chromatographic system** (see *Chromatography (621)*)—The liquid chromatograph is equipped with a 4-mm  $\times$  30-cm column that contains packing L3, a detector that monitors absorption at 254 nm, and a pump capable of providing constant flow up to a minimum of 2000 psi.

**System suitability**—The relative standard deviation for peak response ratios for four replicate injections of *Standard preparation* is not more than 3.0%, and the resolution factor is not less than 3.0.

**Procedure**—Introduce equal volumes of the *Standard preparation* and the *Assay preparation* into the chromatograph (see *Chromatography (621)*), and measure the peak responses obtained. Calculate the quantity, in mg, of  $C_{27}H_{44}O_2 \cdot H_2O$  in the portion of Calcifediol taken by the formula:

$$0.5C(R_u / R_s)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Calcifediol RS in the *Standard preparation*; and  $R_u$  and  $R_s$  are the ratios of the peak responses of calcifediol to testosterone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Calcifediol Capsules

» Calcifediol Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{27}H_{44}O_2 \cdot H_2O$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards (11)**—

USP Calcifediol RS

**Identification**—Transfer the contents of a number of Capsules, equivalent to about 150  $\mu$ g of calcifediol, to a suitable container, add 1 mL of methanol, and shake vigorously for



1 minute. Separate the layers by centrifugation, and transfer as much of the top, methanol layer as possible to a second container. Evaporate this extract to dryness, and dissolve the residue in about 1 mL of chloroform. Proceed as directed under *Thin-layer Chromatographic Identification Test* (201), applying 20  $\mu$ L of this solution and 20  $\mu$ L of a solution containing about the same concentration of USP Calcifediol RS in chloroform, and using a solvent system consisting of 60 parts of cyclohexane and 40 parts of ethyl acetate.

#### Dissolution (711)—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 15 minutes.

**Procedure**—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each capsule shell to rupture.

**Tolerances**—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Internal standard solution**—Dissolve testosterone in ethyl acetate to obtain a solution having a concentration of about 35  $\mu$ g per mL.

**Mobile phase**—Prepare as directed in the Assay under *Calcifediol*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Calcifediol RS in *Internal standard solution*, and dilute quantitatively and stepwise with *Internal standard solution* to obtain a solution having a known concentration of about 7  $\mu$ g of USP Calcifediol RS per mL.

**Assay preparation**—Transfer a number of Calcifediol Capsules to a suitable container. Using a suitable implement, shear open a number of Capsules inside the container. Wash the implement with an accurately measured volume of *Internal standard solution* that will yield a solution having a concentration of about 7  $\mu$ g of calcifediol per mL. Collect the rinsings in the container, and mix to obtain a homogeneous solution of the Capsule contents.

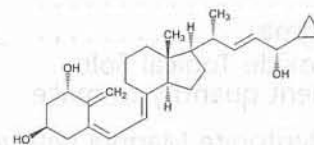
**Chromatographic system and System suitability**—Proceed as directed in the Assay under *Calcifediol*.

**Procedure**—Proceed as directed for *Procedure* in the Assay under *Calcifediol*. Calculate the quantity, in  $\mu$ g, of  $C_{27}H_{44}O_2 \cdot H_2O$  in the portion of Capsule contents taken by the formula:

$$C_U(R_U / R_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Calcifediol RS in the *Standard preparation*;  $V_U$  is the volume, in mL, of *Internal standard solution* taken for the *Assay preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of calcifediol to testosterone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Calcipotriene



$C_{27}H_{40}O_3$  412.60  
9,10-Secochola-5,7,10(19),22-tetraene-1,3,24-triol, 24-cyclopropyl-, (1 $\alpha$ ,3 $\beta$ ,5Z,7E,22E,24S)-; (5Z,7E,22E,24S)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1 $\alpha$ ,3 $\beta$ ,24-triol [112965-21-6].

#### DEFINITION

Calcipotriene contains NLT 97.0% and NMT 102.0% of calcipotriene ( $C_{27}H_{40}O_3$ ), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for Assay.

#### ASSAY

##### • PROCEDURE

Protect solutions containing calcipotriene from light and air. Prepare the *Standard solution* and the *Sample solution* NMT 1 h before use. Prepare the *System suitability solution* daily.

**Buffer:** 1.0 g/L of tris(hydroxymethyl)aminomethane adjusted with phosphoric acid to pH 7.25  $\pm$  0.25

**Mobile phase:** Acetonitrile and Buffer (45:55)

**System suitability solution:** 0.1 mg/mL of USP Calcipotriene RS and 0.01 mg/mL of USP Calcipotriene Related Compound C RS in *Mobile phase*

**Standard solution:** 0.1 mg/mL of USP Calcipotriene RS dissolved in 10% of acetonitrile, then diluted in *Mobile phase*

**Sample solution:** 0.1 mg/mL of Calcipotriene dissolved in 10% of acetonitrile, then diluted in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 264 nm

**Column:** 4.0-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Autosampler temperature:** 4 $^\circ$

**Flow rate:** 1.0 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for calcipotriene related compound C and calcipotriene are 0.94 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between calcipotriene related compound C and calcipotriene

**Relative standard deviation:** NMT 1.0% from the calcipotriene peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of calcipotriene ( $C_{27}H_{40}O_3$ ) in the portion of Calcipotriene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Calcipotriene RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Calcipotriene in the *Sample solution* (mg/mL)



Acceptance criteria: 97.0%–102.0% on the dried basis

## IMPURITIES

### • ORGANIC IMPURITIES BY HPLC

Protect solutions containing calcipotriene from light and air. Prepare the *Standard solution* and the *Sample solution* NMT 1 h before use. Prepare the *System suitability solution* daily.

**Buffer, Mobile phase, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard stock solution:** Use the *Standard solution* in the *Assay*.

**Standard solution:** 0.004 mg/mL of USP Calcipotriene RS in *Mobile phase* from the *Standard stock solution*

**Sample solution:** 0.4 mg/mL of Calcipotriene dissolved in 10% of acetonitrile, then diluted in *Mobile phase*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any impurity in the portion of Calcipotriene taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any impurity from the *Sample solution*

$r_S$  = peak response of calcipotriene from the *Standard solution*

$C_S$  = concentration of USP Calcipotriene RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Calcipotriene in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Calcipotriene related compound C <sup>a</sup>	0.92–0.96	1.00
Calcipotriene	1.00	—
Calcipotriene impurity D <sup>b</sup>	1.13–1.17	1.00
Any individual unspecified impurity	—	0.10
Total impurities	—	2.50

<sup>a</sup> (5E,7E,22E,24S)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1 $\alpha$ ,3 $\beta$ ,24-triol.

<sup>b</sup> (5Z,7E,22E,24R)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1 $\alpha$ ,3 $\beta$ ,24-triol.

### • ORGANIC IMPURITIES BY TLC

Prepare solutions containing calcipotriene in low actinic glassware, and keep from air. Carry out the test as rapidly as possible.

**Diluent:** Chloroform and triethylamine (9:1)

**System suitability solution:** 10 mg/mL of USP Calcipotriene RS in *Diluent*. Heat in a water bath at 60° for 2 h to form precalcipotriene.

**Standard solution 1:** 0.025 mg/mL of USP Calcipotriene RS in *Diluent* (0.25%)

**Standard solution 2:** 0.01 mg/mL of USP Calcipotriene RS in *Diluent* (0.10%)

**Sample solution:** 10 mg/mL of Calcipotriene in *Diluent*

**Developing solvent system:** Methylene chloride and isobutyl alcohol (80:20)

### Chromatographic system

(See *Chromatography* {621}, *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic plate coated with silica gel mixture

**Application volume:** 10  $\mu$ L

**Spray reagent:** Transfer 20 mL of sulfuric acid into a 100-mL volumetric flask, and dilute with alcohol to volume.

### System suitability

**Sample:** *System suitability solution*

### Suitability requirements

**Resolution:** The secondary spot precalcipotriene and principle spot calcipotriene are clearly separated.

### Analysis

**Samples:** *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Develop with *Developing solvent system* until the solvent system has moved 2/3 of the plate from the point of spotting. Remove the plate, and let the plate air-dry. Dry it again at 140° for 10 min followed by spraying the hot plate with the *Spray reagent*. Dry the plate for NMT 1 min at 140°. Examine the plate under UV light at 366 nm.

**Acceptance criteria:** The spot of any impurity in the *Sample solution* is not more intense than the spot of calcipotriene in the appropriate *Standard solution* specified in *Table 2*.

Table 2

Name	Relative Retardation ( $R_{\text{ret}}$ )	Comparison Solution	Acceptance Criteria, NMT (%)
Calcipotriene impurity G <sup>a</sup> and calcipotriene impurity H <sup>b</sup>	0.4	<i>Standard solution 1</i>	0.25
Precalcipotriene <sup>c</sup>	0.9	—	—
Calcipotriene	1.0	—	—
Calcipotriene impurity A <sup>d</sup>	1.2	<i>Standard solution 1</i>	0.25
Any other individual impurity	—	<i>Standard solution 2</i>	0.10

<sup>a</sup> 24,24'-Oxybis[(5Z,7E,22E,24S)-24-cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1 $\alpha$ ,3 $\beta$ -diol].

<sup>b</sup> (5Z,7E,22E,24R)-24-Cyclopropyl-24-[(5Z,7E,22E,24S)-24-cyclopropyl-1 $\alpha$ ,3 $\beta$ -dihydroxy-9,10-secochola-5,7,10(19),22-tetraene-24-yl]oxy-9,10-secochola-5,7,10(19),22-tetraene-1 $\alpha$ ,3 $\beta$ -diol.

<sup>c</sup> (5E,6E,22E,24S)-24-Cyclopropyl-9,10-secochola-5(10),6,22-triene-1 $\alpha$ ,3 $\beta$ ,24-triol.

<sup>d</sup> (5Z,7Z,22E)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-24-one-1 $\alpha$ ,3 $\beta$ -diol.

## SPECIFIC TESTS

### • LOSS ON DRYING

(See *Thermal Analysis* {891}.)

**Sample:** 5 mg

**Analysis:** Heat the *Sample* to 105° at a rate of 10°/min, and hold at 105° for 60 min.

**Acceptance criteria:** NMT 1.0%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers and store at 2°–8° or at –20° or below. Protect from light.

### • USP REFERENCE STANDARDS (11)

USP Calcipotriene RS

USP Calcipotriene Related Compound C RS

(5E,7E,22E,24S)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1 $\alpha$ ,3 $\beta$ ,24-triol.

C<sub>27</sub>H<sub>40</sub>O<sub>3</sub> 412.60



## Calcipotriene Ointment

### DEFINITION

Calcipotriene Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of calcipotriene ( $C_{27}H_{40}O_3$ ), in a suitable ointment base.

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. ULTRAVIOLET ABSORPTION (197U)**  
Buffer, Diluent, Standard stock solution, Standard solution, Sample stock solution, and Sample solution: Proceed as directed in the *Assay*.  
Blank: Dilute 5 mL of tetrahydrofuran with *Diluent* to 25 mL.  
Acceptance criteria: Meets the requirements

### ASSAY

#### • PROCEDURE

The solutions containing calcipotriene are stable up to 24 h at room temperature.

**Mobile phase:** Methanol and water (70:30)

**Buffer:** 132 g/L of monobasic ammonium phosphate in water

**Diluent:** Methanol, *Buffer*, and water (700:3:297)

**Standard stock solution:** 0.1 mg/mL of USP Calcipotriene RS in *Diluent*. Sonicate if necessary.

**Standard solution:** 2 µg/mL of USP Calcipotriene RS prepared as follows. Transfer 5 mL of *Standard stock solution* into a 250-mL volumetric flask, add 50 mL of tetrahydrofuran, and dilute with *Diluent* to volume.

**Sample stock solution:** 0.01 mg/mL of calcipotriene in tetrahydrofuran prepared as follows. Transfer Ointment equivalent to 0.25 mg of calcipotriene into a 25-mL volumetric flask. Add 15 mL of tetrahydrofuran, and sonicate, with intermittent shaking, for 20 min in a cold water bath. Dilute with tetrahydrofuran to volume.

**Sample solution:** 2 µg/mL of calcipotriene prepared as follows. Transfer 5 mL of the *Sample stock solution* into a suitable container. Add 20 mL of *Diluent*, mix, and sonicate for 10 min. Pass through a suitable filter of 0.45-µm pore size. Inject immediately after preparation.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 264 nm

**Column:** 4.6-mm × 15-cm; 3-µm packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 50 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcipotriene ( $C_{27}H_{40}O_3$ ) in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Calcipotriene RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of calcipotriene in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

### IMPURITIES

#### • ORGANIC IMPURITIES

The solutions containing calcipotriene are stable for up to 24 h at room temperature.

**Mobile phase, Buffer, and Diluent:** Proceed as directed in the *Assay*.

**System suitability solution:** 10.0 µg/mL of USP Calcipotriene RS and 0.1 µg/mL of USP Calcipotriene Related Compound C RS in *Diluent*

**Standard stock solution:** 1.0 µg/mL of USP Calcipotriene RS in *Diluent*

**Standard solution:** 0.1 µg/mL of USP Calcipotriene RS prepared as follows. Transfer 1.0 mL of the *Standard stock solution* into a 10-mL volumetric flask, add 1.0 mL tetrahydrofuran, and dilute with *Diluent* to volume.

**Sample solution:** Nominally equivalent to 0.01 mg/mL of calcipotriene prepared as follows. Transfer Ointment equivalent to 0.1 mg of calcipotriene into a glass-stoppered test tube, and add 1 mL of tetrahydrofuran. Sonicate for 20 min with intermittent shaking. Add 9 mL of *Diluent*, and sonicate for 5 min. Shake the test tube vigorously, and then place it in a beaker containing ice cold water for 2–3 min. Pass the liquid portion through a nylon filter of 0.45-µm pore size, and discard the first few mL of the solution.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 264 nm

**Column:** 4.6-mm × 15-cm; 3-µm packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 100 µL

**Run time:** NLT 1.25 times of retention time of the calcipotriene peak

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.2 between calcipotriene related compound C and calcipotriene peaks, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any impurity in the portion of Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any impurity from the *Sample solution*

$r_S$  = peak response of calcipotriene from the *Standard solution*

$C_S$  = concentration of USP Calcipotriene RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of calcipotriene in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.05%.



Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Calcipotriene impurity B <sup>a</sup>	0.86	0.50
Calcipotriene related compound C <sup>b</sup>	0.92	1.00
Calcipotriene	1.0	—
Calcipotriene impurity D <sup>c</sup>	1.31	1.00
Specified unknown impurity	1.8	0.50
Any individual unspecified impurity	—	0.50
Total impurities	—	3.50

<sup>a</sup> (5Z,7Z,22E,24R)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1 $\alpha$ ,3 $\beta$ ,24-triol.

<sup>b</sup> (5E,7E,22E,24S)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1 $\alpha$ ,3 $\beta$ ,24-triol.

<sup>c</sup> (5Z,7E,22E,24R)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1 $\alpha$ ,3 $\beta$ ,24-triol.

### SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count is NMT  $10^2$  cfu/g. The total yeasts and molds count is NMT  $5 \times 10^1$  cfu/g. It meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* species.

- pH (791)**

**Sample solution:** Transfer 1 g of Ointment in a centrifuge test tube. Add 10 mL of water, and heat in a water bath at 60° for 30 min with stirring. Cool to room temperature, and centrifuge at 2500 rpm for 10 min.

**Acceptance criteria:** 5.5–8.5

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature. Do not freeze.
- USP REFERENCE STANDARDS (11)**
  - USP Calcipotriene RS
  - USP Calcipotriene Related Compound C RS
  - (5E,7E,22E,24S)-24-Cyclopropyl-9,10-secochola-5,7,10(19),22-tetraene-1 $\alpha$ ,3 $\beta$ ,24-triol.
  - C<sub>27</sub>H<sub>40</sub>O<sub>3</sub> 412.60

## Calcitonin Salmon

CSNLSCTVLG KLSQELHKLQ TYPRNTGSG TP—NH<sub>2</sub>

C<sub>145</sub>H<sub>240</sub>N<sub>44</sub>O<sub>48</sub>S<sub>2</sub>  
[47931-85-1].

3432 daltons

### DEFINITION

Calcitonin Salmon is a polypeptide that has the same sequence as that of the hormone that regulates calcium metabolism and is secreted by the ultimobranchial gland of salmon. It is produced from either synthetic processes or microbial processes using recombinant DNA (rDNA) technology. The host cell-derived protein content and the host cell- or vector-derived DNA content of Calcitonin Salmon produced from an rDNA process are determined by validated methods. It contains NLT 90.0% and NMT 105.0% of calcitonin salmon, calculated on an acetic acid-free and anhydrous basis. [NOTE—1 mg of acetic acid-free, anhydrous Calcitonin Salmon is equivalent to 6000 USP Calcitonin Salmon Units. 1 USP Calcitonin Salmon Unit = 1 Calcitonin IU.]

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, obtained as directed in the *Assay*.

### ASSAY

- PROCEDURE**

**Solution A:** Dissolve 3.26 g of tetramethylammonium hydroxide pentahydrate in 900 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 100 mL of acetonitrile, and mix.

**Solution B:** Dissolve 1.45 g of tetramethylammonium hydroxide pentahydrate in 400 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 600 mL of acetonitrile, and mix.

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	72	28
30	48	52
32	72	28
55	72	28

**Standard solution:** 1.0 mg/mL of USP Calcitonin Salmon RS in *Solution A*

**System suitability solution:** Prepare a solution in *Solution A* containing about 0.2 mg/mL of USP Calcitonin Salmon Related Compound A RS and 0.2 mg/mL of USP Calcitonin Salmon RS.

**Sample solution:** 1.0 mg/mL of Calcitonin Salmon in *Solution A*

#### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Column temperature:** 65°

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for calcitonin salmon and calcitonin salmon related compound A are 1.0 and 1.15, respectively.]

#### Suitability requirements

**Resolution:** NLT 3 between calcitonin salmon and calcitonin salmon related compound A

**Tailing factor:** NMT 2.5 for calcitonin salmon

**Relative standard deviation:** NMT 3%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of calcitonin salmon

(C<sub>145</sub>H<sub>240</sub>N<sub>44</sub>O<sub>48</sub>S<sub>2</sub>) in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of calcitonin salmon from the *Sample solution*

$r_S$  = peak response of calcitonin salmon from the *Standard solution*

$C_S$  = concentration of USP Calcitonin Salmon RS in the *Standard solution* (corrected for water and acetic acid content) (mg/mL)

$C_U$  = concentration of the *Sample solution* (corrected for water and acetic acid content) (mg/mL)

**Acceptance criteria:** 90.0%–105.0% on an acetic acid-free and anhydrous basis



**OTHER COMPONENTS**• **ACETIC ACID CONTENT** (503)

**Sample solution:** 1 mg/mL of Calcitonin Salmon in *Diluent*, prepared as directed in the chapter  
**Acceptance criteria:** 4%–15%

**IMPURITIES**

**Delete the following:**

• **HEAVY METALS**, *Method II* (231): NMT 50 µg/g (Official 1-

Jan-2018)

• **PROCEDURE: RELATED PEPTIDES AND OTHER RELATED SUBSTANCES****Test 1**

[NOTE—This test is performed on material produced by both chemical synthesis processes and rDNA processes.]

**Solution A, Solution B, Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak area response of each impurity from the *Sample solution*

$r_T$  = sum of the area responses of all the peaks from the *Sample solution*

**Acceptance criteria**

**Individual impurities:** NMT 3.0% of the total area of all peaks

**Total impurities:** NMT 5.0% of the sum of the areas of all the peaks including the principal peak

[NOTE—Disregard any peaks due to the solvent and any peaks whose area is less than 0.1% of the principal peak.]

**Test 2**

[NOTE—This test needs to be performed only on material produced using rDNA technology.]

**Buffer A:** Dissolve 2.72 g of monobasic potassium phosphate in 1000 mL of water.

**Buffer B:** Dissolve 2.72 g of monobasic potassium phosphate and 29.2 g of sodium chloride in 1000 mL of water.

**Buffer C (pH 3.0 citrate buffer):** Dissolve 4.8 g of citric acid in 80 mL of water. Adjust with 1 M sodium hydroxide to a pH of 3.0, and dilute with water to 100.0 mL.

**Solution A:** Acetonitrile and *Buffer A* (15:85). Adjust with 45% (w/w) potassium hydroxide to a pH of 5.0.

**Solution B:** Acetonitrile and *Buffer B* (15:85). Adjust with 45% (w/w) potassium hydroxide to a pH of 4.6.

**Mobile phase:** See *Table 2*.

**Table 2**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	0	100
15	0	100
15.1	100	0
22.1	100	0

**Resolution solution:** Prepare a solution in water containing about 0.5 mg/mL each of USP Calcitonin Salmon RS and USP Calcitonin Salmon Related Compound B RS. To 1 mL of this solution add 100 µL of pH 3.0 citrate buffer.

**Sample solution:** To 1 mL of a 0.5-mg/mL solution of Calcitonin Salmon add 100 mL of *Buffer C*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 20-cm; packing L9

**Flow rate:** 1.2 mL/min

**Injection volume:** 50 µL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for [1,7-bis(3-sulfo-L-alanine)] calcitonin salmon-glycine, [1,7-bis(3-sulfo-L-alanine)] calcitonin salmon, and calcitonin salmon related compound B (calcitonin salmon-glycine) are 0.4, 0.6, and 0.9, respectively; and the retention time for calcitonin salmon is about 9 min.]

**Suitability requirements**

**Resolution:** NLT 3.0 between calcitonin salmon and calcitonin salmon related compound B

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Calcitonin Salmon taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response for each impurity

$r_T$  = sum of the responses of all the peaks

**Acceptance criteria**

**Individual impurities:** See *Table 3*.

**Table 3**

Name	Relative Retention Time	Acceptance Criteria NMT (%)
[1,7-Bis(3-sulfo-L-alanine)] calcitonin salmon-glycine	0.4	0.2
[1,7-Bis(3-sulfo-L-alanine)] calcitonin salmon	0.6	0.2
Calcitonin salmon related compound B	0.9	0.6

**SPECIFIC TESTS**• **AMINO ACID PROFILE**

(See *Biotechnology-Derived Articles—Amino Acid Analysis* (1052).)

[NOTE—This test needs to be performed only on material of synthetic origin. The concentration of amino acids in the *Internal standard solution*, *Standard stock solution*, and *Standard solution* and the amount of material used to prepare the *Sample solution* can be adjusted depending on the method used for amino acid analysis. The concentrations given are based on analysis using *Method 1*.]

**Internal standard solution:** 1 mM solution of γ-aminobutyric acid

**Standard stock solution:** Prepare a mixture containing equimolar amounts of ammonia and the L form of lysine, histidine, arginine, aspartic acid, threonine, serine, proline, valine, glutamic acid, glycine, leucine, and tyrosine, together with half the equimolar amount of L-cysteine, in 0.1 M hydrochloric acid. The final concentration is about 2.5 mM for each amino acid.

**Standard solution:** Transfer 5 mL of the *Internal standard solution* and 2 mL of the *Standard stock solution* into a 50-mL volumetric flask, and dilute with 0.1 M hydrochloric acid to volume.

**Sample solution:** Place 1.5 mg of Calcitonin Salmon into a heavy-wall ignition tube. Add 1.0 mL of 6 N hydrochloric acid, and allow to cool. Immerse the lower half of the tube in a freezing mixture until the contents



are frozen, then evacuate to approximately 10  $\mu$ m of Hg, purge with nitrogen (repeat the evacuation and nitrogen purge three times), and seal the tube while it is under a 10  $\mu$ m of Hg vacuum. Heat for 16 h at 110°–115° in an air oven. Cool, open the tube, dry in a vacuum desiccator, remove the contents, and allow to cool to room temperature. Dissolve in 0.1 M hydrochloric acid. Transfer to a 10-mL volumetric flask, add 1 mL of *Internal standard solution*, and dilute with 0.1 M hydrochloric acid to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Standardize the amino acid analyzer using the *Standard solution*. Inject the *Sample solution* into the amino acid analyzer, and determine the relative proportion of amino acids.

Express the content of each amino acid in moles using an internal standard calibration technique. Calculate the relative proportions of the amino acids by taking as equivalent to one the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, valine, leucine, histidine, arginine, and lysine divided by 20.

**Acceptance criteria:** The requirements are met if the values fall within the limits in *Table 4*.

**Table 4**

Name	Acceptance Criteria
Aspartic acid	1.8–2.2
Glutamic acid	2.7–3.3
Proline	1.7–2.3
Glycine	2.7–3.3
Valine	0.9–1.1
Leucine	4.5–5.3
Histidine	0.9–1.1
Arginine	0.9–1.1
Lysine	1.8–2.2
Serine	3.2–4.2
Threonine	4.2–5.2
Tyrosine	0.7–1.1
Half cystine	1.4–2.1

#### • PEPTIDE MAPPING

(See *Biotechnology-Derived Articles—Peptide Mapping* (1055).)

[NOTE—This test needs to be performed only on material produced using rDNA technology.]

**Solution A:** Water and trifluoroacetic acid (1000:1)

**Solution B:** Acetonitrile, water, and trifluoroacetic acid (800:200:0.85)

**Mobile phase:** See *Table 5*.

**Table 5**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
50	65	35
60	40	60
60.1	0	100
65.1	0	100
65.2	100	0
80.2	100	0

**Trypsin solution:** Freshly prepare a solution containing 0.1 mg/mL of trypsin (previously treated with L-1-tosyl-amido-2-phenylethyl chloromethyl ketone [TPCK] to remove chymotrypsin activity) in water.

**Tris buffer:** 1 M tris(hydroxymethyl) aminomethane and 10 mM calcium chloride. Adjust with hydrochloric acid to a pH of 8.0.

**Stopping solution:** Water and trifluoroacetic acid (1:1)

**Standard solution:** Prepare a 1.0-mg/mL solution of USP Calcitonin Salmon RS. Transfer 1 mL of this solution to a clean vial. Add 100  $\mu$ L of *Tris buffer* and 50  $\mu$ L of *Trypsin solution*. Mix, and incubate at 2°–8° for 16–20 h. Quench the digestion by adding 10  $\mu$ L of *Stopping solution*.

**Sample solution:** 1.0 mg/mL of Calcitonin Salmon.

Transfer 1 mL of this solution to a clean vial. Add 100  $\mu$ L of *Tris buffer* and 50  $\mu$ L of *Trypsin solution*. Mix, and incubate at 2°–8° for 16–20 h. Quench the digestion by adding 10  $\mu$ L of *Stopping solution*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 20  $\mu$ L

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Condition the chromatographic system by running a blank gradient program before injecting the digests.]

**Acceptance criteria:** The chromatographic profile of the *Sample solution* is similar to that of the *Standard solution*.

#### • BIOIDENTITY

**RPMI 1640 with L-glutamine:** Prepare a mixture of the ingredients in the quantities shown in *Table 6* in sufficient water to obtain 1 L of *RPMI 1640 with L-glutamine solution*, and sterilize by filtration.

**Table 6**

Calcium nitrate	100.00 mg
Potassium chloride	400.00 mg
Magnesium sulfate, anhydrous	48.84 mg
Potassium chloride	400 mg
Sodium chloride	6000 mg
Sodium phosphate, dibasic, anhydrous	800 mg
Sodium bicarbonate	2000 mg
Glucose	2000 mg
Glycine	10 mg
L-Arginine	200 mg
L-Asparagine	50 mg
L-Aspartic acid	20 mg
L-Cystine dihydrochloride	65 mg
L-Glutamic acid	20 mg
L-Glutamine	300 mg
L-Histidine	15 mg
L-Hydroxyproline	20 mg
L-Isoleucine	50 mg
L-Leucine	50 mg
L-Lysine hydrochloride	40 mg
L-Methionine	15 mg
L-Phenylalanine	15 mg
L-Proline	20 mg
L-Serine	30 mg
L-Threonine	20 mg
L-Tryptophan	5 mg
L-Tyrosine disodium salt dihydrate	29 mg
L-Valine	20 mg
Biotin	0.2 mg
Choline chloride	3 mg
D-Calcium pantothenate	0.25 mg
Folic acid	1 mg
<i>i</i> -Inositol	35 mg
Niacinamide	1 mg



Table 6 (Continued)

para-Aminobenzoic acid	1 mg
Pyridoxine hydrochloride	1 mg
Riboflavin	0.2 mg
Thiamine hydrochloride	1 mg
Vitamin B <sub>12</sub>	0.005 mg

**Medium A** (growth medium): Using aseptic technique prepare the following tissue culture medium.

Table 7

RPMI 1640 with L-glutamine	500 mL
Fetal bovine serum	50 mL
1 M HEPES	5 mL
Penicillin/streptomycin solution (10,000 IU/mL per 10 mg/mL)	5 mL
Human insulin	10 IU
Hydrocortisone	0.5 mg

**Medium B** (stimulation medium): Dissolve 5 g of bovine serum albumin (BSA) in 500 mL of 2 mM RPMI 1640 with L-glutamine.

**Solution A** (0.2% BSA solution): Dissolve 50 mg of BSA in 25 mL of water. [NOTE—Use within one day.]

**Solution B** (formic acid/BSA solution): Add 25 mL of 0.1 M formic acid and 5 mL of *Solution A* to a 50-mL volumetric flask, dilute with water to volume, and mix. [NOTE—Use within two days.]

**Solution C** (trypsin/EDTA solution): Prepare a sterile filtered solution containing 0.25% trypsin and 0.53 mM EDTA (tetrasodium ethylenediaminetetraacetate).

**Solution D** (Dulbecco's phosphate buffered saline): Dissolve 8 g of sodium chloride, 1.15 g of dibasic sodium phosphate, 0.2 g of monobasic potassium phosphate, 0.2 g of potassium chloride, 0.1 g of calcium chloride, and 0.1 g of magnesium chloride in 1 L of water.

**Standard stock solution:** 20 µg/mL of USP Calcitonin Salmon RS in *Solution B*

**Positive control solution:** Quantitatively dilute the *Standard stock solution* in *Medium B* to obtain a 1-ng/mL solution of USP Calcitonin Salmon RS.

**Negative control solution:** *Medium B*

[NOTE—Prior analysis should be performed to identify the linear portion of the dose-response curve. For example, the *Standard solutions* and *Sample solutions* given below.]

**Standard solution A:** 0.8 ng/mL of USP Calcitonin Salmon RS from the *Standard stock solution* in *Medium B*

**Standard solution B:** 0.4 ng/mL of USP Calcitonin Salmon RS from *Standard solution A* in *Medium B*

**Standard solution C:** 0.2 ng/mL of USP Calcitonin Salmon RS from *Standard solution B* in *Medium B*

**Standard solution D:** 0.1 ng/mL of USP Calcitonin Salmon RS from *Standard solution C* in *Medium B*

**Sample stock solution:** 20 µg/mL of Calcitonin Salmon in *Solution B*

**Sample solution A:** 0.8 ng/mL of Calcitonin Salmon from the *Sample stock solution* in *Medium B*

**Sample solution B:** 0.4 ng/mL of Calcitonin Salmon from *Sample solution A* in *Medium B*

**Sample solution C:** 0.2 ng/mL of Calcitonin Salmon from *Sample solution B* in *Medium B*

**Sample solution D:** 0.1 ng/mL of Calcitonin Salmon from *Sample solution C* in *Medium B*

**Cell culture preparation:** Prepare a cell culture of the human mammary tumor cell line T-47D. Cells are propagated using *Medium A* at 37° and 5% carbon dioxide. The medium is changed every two days, and cells are passaged every 5–9 days using *Solution C* with a 1:4 subculture.

**Cell suspension:** For the test use a cell culture that is 5–9 days old. Remove the cell culture medium from the

flask by aspiration, add 10 mL of *Solution D*, and rock the culture flask to rinse the entire monolayer. Remove the liquid by aspiration, add 2 mL of *Solution C*, spread over the entire monolayer, allow to stand for 3–5 min, and add 10 mL of *Medium A*. Homogenize the cell suspension using a pipet, transfer to a 15-mL polypropylene tube, centrifuge at about 220 × *g* for 5 min, pour off the supernatant, and resuspend the cell pellet in 10 mL of *Medium A*. Count the cells, and adjust the cell density through dilution using *Medium A* to 2.5 × 10<sup>4</sup> cells/mL.

**Procedure:** Place 200 µL of the *Cell suspension* into each well of a 96-well culture plate (the tissue culture plate), and incubate for 18–24 h at 37° and 5% carbon dioxide. Fill each well of an empty, round-bottomed, 96-well culture plate (the prepared plate) with 150 µL of one of the following solutions: *Positive control solution*, *Negative control solution*, *Standard solutions A–D*, and *Sample solutions A–D*, so that each solution fills at least five wells on the prepared plate. After incubation remove the culture medium from the tissue culture plate. Using an 8-channel or 12-channel pipet, rapidly transfer 100 µL of solution from each well of the prepared plate to each well of the tissue culture plate. Incubate for 15 min at ambient temperature, remove the solution from each well, stop stimulation by immediately adding an appropriate cell-lysis buffer, and quantitate cAMP produced within the cells, using a validated kit. Perform the test three times, using three different 96-well culture plates. [NOTE—Some kits include a cell-lysis reagent and a sequestering agent for the cell-lysis reagent. The range of the test kit is between 0.05 and 10 ng/mL of cAMP. The number of cells used in the assay may vary, depending on the validated kit used to quantitate cAMP.]

**Analysis:** Potency is determined by a 3-dose, 6-point parallel-line assay, using standard statistical methods. The calculation is carried out using both the lower three concentrations and the upper three concentrations. For the assay to be valid, the requirements for regression, parallelism, and difference of quadratics must be met. If the requirements for validity are met to the same extent in both assessments (the lower and the higher assessments) the final result is determined from the concentration range that shows the higher value when the common slope is divided by the root mean square error.

**Acceptance criteria:** Combine the three potency values by using an unweighted mean on the log scale. Determine a 95% confidence interval in the log scale using standard statistical methods. Convert the average and confidence interval to the potency scale using antilogs to obtain a geometric mean and its confidence interval. The potency levels determined from all three performances of the test are valid if the data analysis indicates the three determinations to be homogeneous, and the confidence interval is fully contained within 64% and 156% of the geometric mean. If the confidence interval requirement is not met, additional assays may be performed to increase the number of assays and make the confidence interval narrower. The determination of whether it meets the identity requirement should be done only after the confidence interval requirement is met. The acceptance criterion for identity is that the geometric mean is within 80% and 125% of the Assay value.

• **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62)**

**Sample:** 25 mg

**Acceptance criteria:** The total aerobic microbial count is NMT 10<sup>2</sup> cfu/g, and the total combined molds and yeasts count is NMT 10<sup>2</sup> cfu/g.

• **STERILITY TESTS (71):** Where the label states that Calcitonin Salmon is sterile, it meets the requirements



when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

- **WATER DETERMINATION, Method Ic (921):** NMT 10%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store in a refrigerator or maintain in a frozen state, protected from light.
- **LABELING:** The labeling states that the material is synthetic or of recombinant DNA origin.
- **USP REFERENCE STANDARDS (11)**
  - USP Calcitonin Salmon RS
  - $C_{145}H_{240}N_{44}O_{48}S_2$  3432 daltons
  - USP Calcitonin Salmon Related Compound A RS
  - N-Acetyl-cys<sup>1</sup>-calcitonin salmon.
  - USP Calcitonin Salmon Related Compound B RS
  - (calcitonin salmon-glycine)
  - Calcitonin salmon-glycine.

### Calcitonin Salmon Injection

#### DEFINITION

Calcitonin Salmon Injection is a sterile solution of Calcitonin Salmon in a suitable diluent. Each mL of Calcitonin Salmon Injection possesses an activity of NLT 80% and NMT 120% of that stated on the label.

#### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

**Solution A:** Dissolve 3.26 g of tetramethylammonium hydroxide pentahydrate in 900 mL of water, add 100 mL of acetonitrile, and mix. Adjust with phosphoric acid to a pH of 2.5, pass through a filter of 0.5- $\mu$ m or finer pore size, and degas.

**Solution B:** Dissolve 1.45 g of tetramethylammonium hydroxide pentahydrate in 400 mL of water, add 600 mL of acetonitrile, and mix. Adjust with phosphoric acid to a pH of 2.5, pass through a filter of 0.5- $\mu$ m or finer pore size, and degas.

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	72	28
30	48	52
32	72	28
55	72	28

**System suitability solution:** Prepare a solution in *Solution A* containing about 0.2 mg/mL of USP Calcitonin Salmon Related Compound A RS and 0.2 mg/mL of USP Calcitonin Salmon RS. Take 0.1 mL of this solution, add 0.9 mL of *Solution A*, and mix.

**Standard stock solution:** 1.0 mg/mL of USP Calcitonin Salmon RS in *Solution A*

**Standard solution:** 0.1 mg/mL of USP Calcitonin Salmon RS from *Standard stock solution* diluted with *Solution A*

**Sample solution:** Use the solution from an undiluted Injection vial.

#### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Column temperature:** 65°

**Flow rate:** 1 mL/min

**Injection volume:** 200  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for calcitonin salmon and calcitonin salmon related compound A are 1.0 and 1.15, respectively.]

#### Suitability requirements

**Resolution:** NLT 3 between calcitonin salmon and calcitonin salmon related compound A

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 3%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the potency, in USP Calcitonin Salmon Units/mL, in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times C_s$$

$r_u$  = peak area from the *Sample solution*

$r_s$  = peak area from the *Standard solution*

$C_s$  = concentration of USP Calcitonin Salmon RS in the *Standard solution* (USP Calcitonin Salmon Units/mL)

**Acceptance criteria:** 80%–120%

#### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.625 USP Endotoxin Units/USP Calcitonin Salmon Unit
- **STERILITY TESTS (71):** Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **PH (791):** 3.9–4.5
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS (1):** Meets the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Avoid freezing. Store in a refrigerator.
- **LABELING:** Label it to indicate the activity in USP Calcitonin Salmon Units/mL. The labeling states that the material is synthetic. Label it to state that it is to be stored in a refrigerator, and that freezing is to be avoided.
- **USP REFERENCE STANDARDS (11)**
  - USP Calcitonin Salmon RS
  - USP Calcitonin Salmon Related Compound A RS
  - N-Acetyl-cys<sup>1</sup>-calcitonin.
  - $C_{146}H_{243}N_{44}O_{49}S_2$  3463
  - USP Endotoxin RS

### Calcitonin Salmon Nasal Solution

#### DEFINITION

Calcitonin Salmon Nasal Solution is a solution of Calcitonin Salmon in a suitable diluent. It contains suitable preservatives, and is packaged in a form suitable for nasal administration so that the required dosage can be controlled as required. Each mL of Calcitonin Salmon Nasal Solution possesses an activity of NLT 80% and NMT 115% of that stated on the label.



**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Solution A:** 3.26 mg/mL of tetramethylammonium hydroxide pentahydrate in water and acetonitrile (9:1). Adjust with phosphoric acid to a pH of 2.5.

**Solution B:** 1.45 mg/mL of tetramethylammonium hydroxide pentahydrate in acetonitrile and water (3:2). Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	72	28
30	48	52
32	72	28
55	72	28

**Diluent:** 7.5 mg/mL of sodium chloride, 2 mg/mL of sodium acetate, and 2 mg/mL of glacial acetic acid in water

**System suitability stock solution:** Prepare a solution in *Solution A* containing about 0.2 mg/mL of USP Calcitonin Salmon Related Compound A RS and 0.2 mg/mL of USP Calcitonin Salmon RS.

**System suitability solution:** *System suitability stock solution* and *Solution A* (1:9)

**Standard stock solution:** 1.0 mg/mL of USP Calcitonin Salmon RS in *Solution A*

**Standard solution:** 0.1 mg/mL of USP Calcitonin Salmon RS from the *Standard stock solution* in *Solution A*

**Sample solution:** Nasal Solution in *Diluent* (1:9)

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Column temperature:** 65°

**Flow rate:** 1 mL/min

**Injection volume:** 200 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for calcitonin salmon and calcitonin salmon related compound A are 1.0 and 1.15, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between calcitonin salmon and calcitonin salmon related compound A, *System suitability solution*

**Tailing factor:** NMT 2.5, *Standard solution*

**Relative standard deviation:** NMT 3.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcitonin salmon (C<sub>145</sub>H<sub>240</sub>N<sub>44</sub>O<sub>48</sub>S<sub>2</sub>) in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak area from the *Sample solution*  
 $r_S$  = peak area from the *Standard solution*  
 $C_S$  = concentration of USP Calcitonin Salmon RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of calcitonin salmon in the *Sample solution* (mg/mL)

Acceptance criteria: 80%–115%

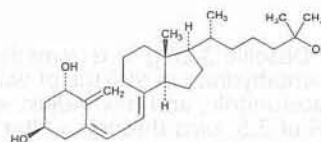
**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count is NMT 10<sup>2</sup> cfu/g, and the total combined molds and yeasts count is NMT 5 × 10<sup>1</sup> cfu/g. It meets the requirements for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

- **pH** (791): 3.5–4.5

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in containers suitable for spraying the contents into the nasal cavities in a controlled individualized dosage. Store unopened containers in a refrigerator and opened containers at room temperature.
- **LABELING:** Label it to indicate that it is for intranasal administration only. The labeling also states that it has been prepared either with Calcitonin Salmon of synthetic origin or Calcitonin Salmon of rDNA origin. Label it to state that it is to be stored in a refrigerator, and that freezing is to be avoided. Label it to indicate the activity in USP Calcitonin Salmon Units/mL.
- **USP REFERENCE STANDARDS** (11)  
 USP Calcitonin Salmon RS  
 USP Calcitonin Salmon Related Compound A RS  
 N-Acetyl-cys<sup>1</sup>-calcitonin.  
 C<sub>146</sub>H<sub>243</sub>N<sub>44</sub>O<sub>49</sub>S<sub>2</sub> 3463

**Calcitriol**

C<sub>27</sub>H<sub>44</sub>O<sub>3</sub> 416.64

9,10-Secocholesta-5,7,10(19)-triene-1,3,25-triol, (1 $\alpha$ ,3 $\beta$ ,5 $\zeta$ ,7 $\beta$ )-

(5 $\zeta$ ,7 $\beta$ )-9,10-Secocholesta-5,7,10(19)-triene-1 $\alpha$ ,3 $\beta$ ,25-triol [32222-06-3].

Monohydrate 434.65 [77326-95-5].

» Calcitriol is anhydrous or contains one molecule of hydration. The anhydrous form contains not less than 97.0 percent and not more than 103.0 percent of C<sub>27</sub>H<sub>44</sub>O<sub>3</sub>, calculated on the solvent-free basis. The monohydrate form contains not less than 97.0 percent and not more than 103.0 percent of C<sub>27</sub>H<sub>44</sub>O<sub>3</sub>, calculated on the anhydrous basis.

**Caution**—Care should be taken to prevent inhaling particles of calcitriol and exposing the skin to it.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store as per labeling instructions.

**Labeling**—Where it is a monohydrate form, the label so indicates.

**USP Reference standards** (11)—

USP Calcitriol RS

**Identification**—

**A:** *Infrared Absorption* (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the



chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water Determination, Method 1c** (921) (where it is labeled as a monohydrate): between 3.5% and 5.5%.

**Chromatographic purity**—[NOTE—Carry out the procedure as rapidly as possible, avoiding unnecessary exposure of solutions to light and air.]

*Tris buffer solution*, *Mobile phase*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay*.

*Test solution*—Prepare as directed for *Assay preparation*.

*Procedure*—Inject a volume (about 50  $\mu$ L) of the *Test solution* into the chromatograph, record the chromatograms for at least two times the retention time of the calcitriol peak, identify the impurities listed in *Table 1*, and measure the peak responses. Calculate the percentage of any individual impurity in the portion of Calcitriol taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response of any individual peak other than the main calcitriol peak and the pre-calcitriol peak; and  $r_s$  is the sum of the responses of all the peaks: in addition to not exceeding the limits in *Table 1*, not more than 1.0% of total impurities is found. Disregard any peak less than 0.1%.

Table 1

Name	Relative Retention Time	Limit (%)
Triazoline adduct of pre-calcitriol	0.43	0.1
<i>trans</i> -Calcitriol <sup>1</sup>	0.96	0.25
1 $\beta$ -Calcitriol <sup>2</sup>	1.15	0.1
Methylene calcitriol <sup>3</sup>	1.5	0.25
Any other individual unidentified impurity	—	0.1

<sup>1</sup>(5E,7E)-9,10-secocholesta-5,7,10(19)-triene-1 $\alpha$ ,3 $\beta$ ,25-triol

<sup>2</sup>(5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-1 $\beta$ ,3 $\beta$ ,25-triol

<sup>3</sup>(5Z,7E)-1 $\alpha$ ,3 $\beta$ -dihydroxy-17-((R)-7-hydroxy-7-methyloctan-2-yl)-9,10-secoandrosta-5,7,10(19)-triene

**Assay**—[NOTE—Carry out the procedure as rapidly as possible, avoiding unnecessary exposure of solutions to light and air.]

*Tris buffer solution*—Dissolve 1.0 g of tris(hydroxymethyl)aminomethane in 900 mL of water, adjust with phosphoric acid to a pH of 7.0 to 7.5, dilute with water to make 1000 mL, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of acetonitrile and *Tris buffer solution* (55:45). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Transfer an accurately weighed quantity of USP Calcitriol RS to a suitable volumetric flask, dissolve first in acetonitrile (without heating), using 55% of the final volume, then dilute with *Tris buffer solution* to volume, and mix to obtain a solution having a known concentration of about 100  $\mu$ g of calcitriol per mL. [NOTE—Allow the solution to warm up to room temperature before diluting with *Tris buffer solution* to final volume.]

*System suitability solution*—Heat 2.0 mL of the *Standard preparation* at 80° for 30 minutes.

*Assay preparation*—Transfer an accurately weighed quantity of Calcitriol to a suitable volumetric flask, dissolve first in acetonitrile (without heating), using 55% of the final volume, then dilute with *Tris buffer solution* to volume, and mix to obtain a solution having a known concentration of about 100  $\mu$ g of calcitriol per mL. [NOTE—Allow the solution to warm up to room temperature before diluting with *Tris buffer solution* to final volume.]

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L7. The flow rate is about 1 mL per minute. The column temperature is maintained at 40°. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for pre-calcitriol and 1.0 for calcitriol; and the resolution,  $R$ , between pre-calcitriol and calcitriol is not less than 3.5. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 10,000 theoretical plates; and the relative standard deviation for replicate injections is not more than 1.0%.

*Procedure*—Separately inject equal volumes (about 50  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the calcitriol and pre-calcitriol peaks. Calculate the percentage of  $C_{27}H_{44}O_3$  in the portion of Calcitriol taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which  $C_s$  and  $C_u$  are the concentrations, in  $\mu$ g per mL, of calcitriol in the *Standard preparation* and the *Assay preparation*, respectively; and  $r_u$  and  $r_s$  are the sums of the calcitriol and pre-calcitriol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Calcitriol Injection

» Calcitriol Injection is a sterile solution of Calcitriol. It contains an amount of Calcitriol equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of calcitriol ( $C_{27}H_{44}O_3$ ). It contains no antimicrobial agents.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light. Store at controlled room temperature.

**USP Reference standards** (11)—

USP Calcitriol Solution RS

USP Endotoxin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 100 USP Endotoxin Units per  $\mu$ g of calcitriol.

**pH** (791): between 5.9 and 8.0, determined on a portion to which, if necessary, 0.30 mL of saturated potassium chloride solution has been added for each 100 mL of Injection.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—[NOTE—Avoid unnecessary exposure of solutions to light or air.]

*Mobile phase*—Prepare a filtered and degassed mixture of methanol and water (74:26). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)) so that the retention time for calcitriol is not less than 20 minutes.

*Standard preparation*—Transfer 3.0 mL of USP Calcitriol Solution RS, equilibrated to room temperature, to a container; add 3.0 mL of water; and mix.

*Assay preparation*—Transfer an accurately measured volume of Injection, equivalent to about 3  $\mu$ g of calcitriol, to a



container; add a sufficient amount of water to dilute to a total volume of 3.0 mL; add 3.0 mL of methanol; and mix.

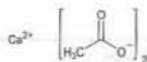
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 264-nm detector, a 4.6-mm × 4.5-cm guard column that contains 5-μm packing L1, and a 4.6-mm × 7.5-cm analytical column that contains 3-μm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*; the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 100 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in μg, of calcitriol (C<sub>27</sub>H<sub>44</sub>O<sub>3</sub>) in each mL of the injection taken by the formula:

$$C(r_u / r_s)$$

in which C is the concentration, in μg per mL, of calcitriol in the USP Calcitriol Solution RS; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Calcium Acetate



C<sub>4</sub>H<sub>6</sub>CaO<sub>4</sub> 158.17  
Acetic acid, calcium salt;  
Calcium acetate [62-54-4].

### DEFINITION

Calcium Acetate contains NLT 99.0% and NMT 100.5% of calcium acetate (C<sub>4</sub>H<sub>6</sub>CaO<sub>4</sub>), calculated on the anhydrous basis.

### IDENTIFICATION

- A. IDENTIFICATION TESTS—GENERAL**, *Calcium* (191) and *Acetate* (191)

Sample solution: 50 mg/mL

Acceptance criteria: Meets the requirements

### ASSAY

#### PROCEDURE

Sample: 300 mg

**Analysis:** Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 7.909 mg of calcium acetate (C<sub>4</sub>H<sub>6</sub>CaO<sub>4</sub>).

Acceptance criteria: 99.0%–100.5% on the anhydrous basis

### IMPURITIES

- ARSENIC**, *Method I* (211): NMT 3 ppm

- CHLORIDE AND SULFATE**, *Chloride* (221)

Standard: 0.70 mL of 0.020 N hydrochloric acid

Sample: 1.0 g

Acceptance criteria: 0.05%

- CHLORIDE AND SULFATE**, *Sulfate* (221)

Standard: 0.15 mL of 0.020 N sulfuric acid

Sample: 0.25 g

Acceptance criteria: 0.06%

### Delete the following:

- HEAVY METALS**, *Method I* (231)

**Test preparation:** Dissolve 0.8 g of Calcium Acetate in 20 mL of water. Add 3.0 mL of glacial acetic acid, dilute with water to 25 mL, and adjust with glacial acetic acid to a pH of 3.8–4.0, measured with a pH meter.

**Monitor preparation:** Prepare as directed for the *Test preparation*, 2.0 mL of *Standard Lead Solution* being added.

Acceptance criteria: NMT 25 ppm • (Official 1-Jan-2018)

- LEAD** (251): NMT 10 ppm

- LIMIT OF ALUMINUM**

[NOTE—Use where it is labeled as intended for parenteral use or for use in hemodialysis or peritoneal dialysis.]

**Buffer:** Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, and dilute with water to 250 mL.

**Aluminum standard solution:** 1.0 μg/mL of aluminum. Prepare as directed for *Standard Preparations in Aluminum* (206).

**Standard solution:** Prepare a solution containing 2.0 mL of *Aluminum standard solution*, 5 mL of *Buffer*, and 48 mL of water, and extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume.

**Sample solution:** Dissolve 1.0 g of Calcium Acetate in 50 mL of water, and add 5 mL of *Buffer*. Extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume.

**Blank solution:** Prepare a solution containing 50 mL of water and 5 mL of *Buffer*. Extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume.

### Instrumental conditions

(See *Fluorescence Spectroscopy* (853).)

Mode: Fluorescence

Excitation wavelength: 392 nm

Emission wavelength: 518 nm

### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank solution*

Use the *Blank solution* to zero the instrument.

Acceptance criteria: 2 ppm; the fluorescence of the *Sample solution* is NMT that of the *Standard solution*.

- LIMIT OF BARIUM**

[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis.]

**Barium chloride solution:** 500 μg/mL of barium in water from anhydrous barium chloride

**Buffer:** Ammonium sulfate solution (1 in 10)

**Standard solution:** To a tube add 1 g of ammonium acetate, 2 mL of 1 N hydrochloric acid, 3.0 mL of *Barium chloride solution*, and sufficient water to bring the volume to 40 mL.

**Sample stock solution:** 250 mg/mL of Calcium Acetate and 25 mg/mL of ammonium acetate in 1 N hydrochloric acid. The pH of this solution is 4.5–5.5. Filter, and cover the solution.

**Sample solutions:** To four separate tubes add 1.0, 1.5, 2.0, and 2.5 mL of *Barium chloride solution*. To each tube add a sufficient volume of the *Sample stock solution* to bring the volume to 40 mL.



**Analysis:** To the *Sample solutions* and the *Standard solution* add, with brisk stirring, 3.0 mL of *Buffer*, and allow to stand for 20 min.

**Acceptance criteria:** The *Sample solutions* containing 1.0 and 1.5 mL of *Barium chloride solution* remain clear or are only faintly turbid. The *Sample solution* containing 2.0 mL of *Barium chloride solution* is not more turbid than the *Standard solution*.

#### • LIMIT OF FLUORIDE

[NOTE—Prepare and store all solutions in plastic containers.]

**Buffer:** 294 mg/mL of sodium citrate dihydrate in water

**Standard stock solution:** 1.11 mg/mL of USP Sodium Fluoride RS in water

**Standard solution:** Combine 20.0 mL of *Standard stock solution* with 50.0 mL of *Buffer*, and dilute with water to 100.0 mL. Equivalent to 100 µg/mL of fluoride

**Sample solution:** Transfer 2.0 g of Calcium Acetate to a beaker containing a plastic-coated stirring bar. Add 20.0 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer* and sufficient water to make 100 mL.

**Electrode system:** Use a fluoride-specific, ion-indicating electrode and a silver-silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of  $\pm 0.2$  mV (see pH <791>).

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Transfer 50.0 mL of *Buffer* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of the *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV. Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the standard response line determine the concentration, *C*, in µg/mL, of fluoride ion in the *Sample solution*. Calculate the amount of fluoride (ppm) in the sample taken by multiplying *C* by 50.

**Acceptance criteria:** 50 ppm

#### • LIMIT OF MAGNESIUM

[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

**Standard stock solution:** 1000 µg/mL of magnesium in 1 N nitric acid from magnesium oxide

**Standard solution:** 5.0 µg/mL of magnesium from the *Standard stock solution*

**Sample solution:** 2 mg/mL of Calcium Acetate

**Linearity solution A:** Dilute 20.0 mL of the *Sample solution* with water to 25.0 mL (0 µg/mL of magnesium).

**Linearity solution B:** Dilute 2.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (0.4 µg/mL of magnesium).

**Linearity solution C:** Dilute 4.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (0.8 µg/mL of magnesium).

#### Instrumental conditions

(See *Atomic Absorption Spectroscopy* <852>.)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 285.2 nm

**Flame:** Air-acetylene

**Lamp:** Magnesium hollow-cathode

**Blank:** Water

#### Analysis

**Samples:** *Linearity solutions* A, B, and C

Plot the absorbances of the *Linearity solutions* versus their content of magnesium (0, 0.4, and 0.8 µg/mL), draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of magnesium in the *Sample solution*.

Calculate the percentage of magnesium in the sample by multiplying this value by 0.0625.

**Acceptance criteria:** NMT 0.05%

#### • LIMIT OF NITRATE

**Sample solution:** 100 mg/mL of Calcium Acetate in water

**Analysis:** To 10 mL of the *Sample solution* add 5 mg of sodium chloride, 0.05 mL of indigo carmine TS, and, with stirring, 10 mL of nitrogen-free sulfuric acid.

**Acceptance criteria:** The blue color persists for NLT 10 min.

#### • LIMIT OF POTASSIUM

[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

**Standard stock solution:** 23.84 mg/mL of potassium chloride, using potassium chloride previously dried at 105° for 2 h, equivalent to 12.5 mg/mL of potassium

**Standard solution:** 31.25 µg/mL of potassium from the *Standard stock solution*

**Sample solution:** 12.5 mg/mL of Calcium Acetate

**Linearity solution A:** Dilute 20.0 mL of the *Sample solution* with water to 25.0 mL (0 µg/mL of potassium).

**Linearity solution B:** Dilute 2.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (2.5 µg/mL of potassium).

**Linearity solution C:** Dilute 4.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (5.0 µg/mL of potassium).

#### Instrumental conditions

(See *Atomic Absorption Spectroscopy* <852>.)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 766.7 nm

**Lamp:** Potassium hollow-cathode

**Flame:** Air-acetylene

**Blank:** Water

#### Analysis

**Samples:** *Linearity solutions* A, B, and C

Plot the absorbances of the *Linearity solutions* versus their content of potassium (0, 2.5, and 5.0 µg/mL), draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of potassium in the *Sample solution*.

Calculate the percentage of potassium in the sample by multiplying this value by 0.01.

**Acceptance criteria:** NMT 0.05%

#### • LIMIT OF SODIUM

[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and the *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

**Standard stock solution:** 25.42 mg/mL of sodium chloride, using sodium chloride previously dried at 105° for 2 h, equivalent to 10.0 mg/mL of sodium

**Standard solution:** 250 µg/mL of sodium from the *Standard stock solution*



**Sample solution:** 10 mg/mL of Calcium Acetate  
**Linearity solution A:** Dilute 20.0 mL of the *Sample solution* with water to 25.0 mL (0 µg/mL of sodium).

**Linearity solution B:** Dilute 2.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (20 µg/mL of sodium).

**Linearity solution C:** Dilute 4.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (40 µg/mL of sodium).

#### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 589.0 nm

**Lamp:** Sodium hollow-cathode

**Flame:** Air-acetylene

**Blank:** Water

#### Analysis

**Samples:** *Linearity solutions A, B, and C*

Plot the absorbances of the *Linearity solutions* versus their content of sodium (0, 20, and 40 µg/mL), draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of sodium in the *Sample solution*.

Calculate the percentage of sodium in the sample by multiplying this value by 0.0125.

**Acceptance criteria:** NMT 0.5%

#### • LIMIT OF STRONTIUM

[NOTE—Use where it is labeled as intended for use in hemodialysis or peritoneal dialysis. The *Standard solution* and *Sample solutions* may be modified, if necessary, to obtain solutions of suitable concentrations, adaptable to the linear or working range of the instrument.]

**Standard stock solution:** 2.45 mg/mL of strontium acetate in water, equivalent to 1000 µg/mL of strontium

**Standard solution:** 50.0 µg/mL of strontium from the *Standard stock solution*

**Sample solution:** 20 mg/mL of Calcium Acetate

**Linearity solution A:** Dilute 20.0 mL of the *Sample solution* with water to 25.0 mL (0 µg/mL of strontium).

**Linearity solution B:** Dilute 2.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (4 µg/mL of strontium).

**Linearity solution C:** Dilute 4.0 mL of the *Standard solution* and 20.0 mL of the *Sample solution* with water to 25.0 mL (8 µg/mL of strontium).

#### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 460.7 nm

**Lamp:** Strontium hollow-cathode

**Flame:** Nitrous oxide-acetylene

**Blank:** Water

#### Analysis

**Samples:** *Linearity solutions A, B, and C*

Plot the absorbances of the *Linearity solutions* versus their content of strontium (0, 4, and 8 µg/mL), draw the straight line best fitting the three points, and extrapolate the line until it intercepts the concentration axis. From the intercept determine the amount, in µg/mL, of strontium in the *Sample solution*.

Calculate the percentage of strontium in the sample by multiplying this value by 0.00625.

**Acceptance criteria:** NMT 0.05%

#### • READILY OXIDIZABLE SUBSTANCES

**Sample solution:** 20 mg/mL of Calcium Acetate in boiling water

**Analysis:** Add a few glass beads to 100 mL of the *Sample solution*, 6 mL of 10 N sulfuric acid, and 0.3 mL of 1 N potassium permanganate. Mix, boil gently for 5 min, and allow the precipitate to settle.

**Acceptance criteria:** The pink color in the supernatant is not completely discharged.

#### SPECIFIC TESTS

##### • PH (791)

**Sample solution:** 50 mg/mL

**Acceptance criteria:** 6.3–9.6

##### • WATER DETERMINATION, Method I (921)

**Sample:** 0.100 g

**Analysis:** Proceed as directed in the chapter, adding 2 mL of glacial acetic acid to the titration vessel in addition to the methanol.

**Acceptance criteria:** NMT 7.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** Where Calcium Acetate is intended for use in hemodialysis or peritoneal dialysis, it is so labeled.

• **USP REFERENCE STANDARDS (11)**

USP Sodium Fluoride RS

## Calcium Acetate Tablets

#### DEFINITION

Calcium Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium acetate ( $C_4H_6CaO_4$ ).

#### IDENTIFICATION

• **IDENTIFICATION TESTS—GENERAL, Calcium (191) and Acetate (191)**

**Sample solution:** 100 mg/mL of calcium acetate from powdered Tablets

**Acceptance criteria:** Meet the requirements

#### ASSAY

##### • PROCEDURE

**Sample:** Amount equivalent to 300 mg of calcium acetate from NLT 20 powdered Tablets

**Analysis:** Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, add 30 mL of 0.05 M edetate disodium VS from a 50-mL buret, and add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue. Continue the titration with the 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 7.909 mg of calcium acetate ( $C_4H_6CaO_4$ ).

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

##### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrometry

**Analytical wavelength:** 422.8 nm

**Standard solution:** Calcium at a known concentration in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration that is similar to the *Standard solution*.

**Acceptance criteria:** NLT 80% (Q) of the labeled amount of calcium acetate ( $C_4H_6CaO_4$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • LIMIT OF ALUMINUM

**Buffer:** Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, and dilute with water to 250 mL.



**Aluminum standard solution** 1.0 µg/mL of aluminum. Prepare as directed for *Standard Preparation* under *Aluminum* (206).

**Standard solution:** Prepare a solution containing 2.0 mL of *Aluminum standard solution*, 5 mL of *Buffer*, and 48 mL of water, and extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask, and dilute the combined extracts with chloroform to volume.

**Sample solution:** Dissolve a portion of powdered Tablets (NLT 10) equivalent to 1.0 g of calcium acetate in 50 mL of water, and add 5 mL of *Buffer*. Extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask, and dilute the combined extracts with chloroform to volume.

**Blank solution:** Prepare a solution containing 50 mL of water and 5 mL of *Buffer*. Extract this solution with successive portions of 10, 10, and 5 mL of 0.5% 8-hydroxyquinoline in chloroform. Combine the chloroform extracts in a 50-mL volumetric flask, and dilute the combined extracts with chloroform to volume.

#### Instrumental conditions

(See *Fluorescence Spectroscopy* (853).)

**Mode:** Fluorescence

**Excitation wavelength:** 392 nm

**Emission wavelength:** 518 nm

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank solution*. Use the *Blank solution* to zero the instrument.

**Acceptance criteria:** The fluorescence of the *Sample solution* does not exceed that of the *Standard solution* (NMT 2 ppm).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## Calcium Ascorbate

$C_{12}H_{14}CaO_{12} \cdot 2H_2O$  426.34

#### DEFINITION

Calcium Ascorbate contains NLT 98.0% and NMT 101.0% of calcium ascorbate dihydrate ( $C_{12}H_{14}CaO_{12} \cdot 2H_2O$ ), calculated on the as-is basis.

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** A 100 mg/mL solution meets the requirements.
- **B.** A 100 mg/mL solution decolorizes a 100 mg/mL solution of dichlorophenol-indophenol.
- **C. INFRARED ABSORPTION (197M)**

#### ASSAY

##### • PROCEDURE

**Sample:** 300 mg of Calcium Ascorbate

**Blank:** 50 mL of water

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N iodine VS

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* into a 250-mL conical flask, add 50 mL of water, and mix to dissolve. Immediately titrate with the *Titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform a *Blank* determination.

Calculate the percentage of calcium ascorbate dihydrate ( $C_{12}H_{14}CaO_{12} \cdot 2H_2O$ ) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F]/W\} \times 100$$

$V_S$  = Titrant volume consumed by the *Sample* (mL)

$V_B$  = Titrant volume consumed by the *Blank* (mL)

$N$  = Titrant normality (mEq/mL)

$F$  = equivalency factor, 106.6 mg/mEq

$W$  = Sample weight (mg)

**Acceptance criteria:** 98.0%–101.0% on the as-is basis

#### IMPURITIES

- **ARSENIC, Method I (211):** NMT 3 µg/g

#### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 10 ppm (Official 1-

Jan-2018)

#### • LIMIT OF FLUORIDE

[NOTE—Prepare and store all solutions in plastic containers.]

**Buffer solution:** 294 mg/mL of sodium citrate dihydrate in water

**Standard stock solution:** 1.1052 mg/mL of USP Sodium Fluoride RS in water

**Standard solution:** Transfer 20.0 mL of *Standard stock solution* to a 100-mL volumetric flask containing 50.0 mL of *Buffer solution*, dilute with water to volume, and mix. Each mL of *Standard solution* contains 100 µg of fluoride ion.

**Sample solution:** Transfer 2.0 g of Calcium Ascorbate to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer solution* and sufficient water to make 100 mL.

**Electrode system:** Use a fluoride-specific ion-indicating electrode and a silver-silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ± 0.2 mV (see pH (791)).

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Standard response line:** Transfer 50.0 mL of *Buffer solution* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential, in mV.

Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line* determine the concentration,  $C$  (in µg/mL), of fluoride ion in the *Sample solution*.

Calculate the content of fluoride in the portion of Calcium Ascorbate taken:

$$\text{Result} = (C \times V)/W$$

$C$  = concentration of fluoride ion in the *Sample solution* (µg/mL), obtained from the *Standard response line*

$V$  = volume of the *Sample solution* (mL)

$W$  = weight of Calcium Ascorbate taken to prepare the *Sample solution* (g)



Acceptance criteria: NMT 10 ppm

### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation (7815)**  
Sample solution: 50 mg/mL in carbon dioxide-free water [NOTE—Perform measurements immediately after preparation.]  
Acceptance criteria: +95° to +97°
- **pH (791)**  
Sample solution: 100 mg/mL  
Acceptance criteria: 6.8–7.4
- **LOSS ON DRYING (731):** Dry 3 g at 105° for 2 h; it loses NMT 0.1% of its weight.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Calcium Ascorbate RS  
USP Sodium Fluoride RS

## Calcium Carbonate

CaCO<sub>3</sub> 100.09  
Carbonic acid, calcium salt (1:1);  
Calcium carbonate (1:1) [471-34-1].

### DEFINITION

Calcium Carbonate, dried at 200° for 4 h, contains calcium equivalent to NLT 98.0% and NMT 100.5% of calcium carbonate (CaCO<sub>3</sub>).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** The addition of acetic acid to it produces effervescence (presence of carbonate), and the resulting solution, after boiling, meets the requirements of the tests.

### ASSAY

- **TITRIMETRY (541)**  
Sample: 200 mg of Calcium Carbonate, previously dried at 200° for 4 h  
Blank: 100 mL of water and 15 mL of 1 N sodium hydroxide  
Titrimetric system  
(See *Titrimetry* (541).)  
Mode: Direct titration  
Titrant: 0.05 M edetate disodium VS  
Indicator: 300 mg of hydroxy naphthol blue  
Endpoint detection: Visual, change to distinct blue  
Analysis: Transfer the Sample to a 250-mL beaker. Moisten thoroughly with a few mL of water, and add, dropwise, sufficient 3 N hydrochloric acid to dissolve. Add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue. Titrate with the Titrant. Calculate the percentage of calcium carbonate (CaCO<sub>3</sub>) in the Sample taken:

$$\text{Result} = [(V - B) \times M \times F \times 100] / W$$

V = Sample titrant volume (mL)

B = Blank titrant volume (mL)

M = titrant molarity (mmol/mL)

F = equivalency factor, 100.09 mg/mmol

W = weight of the Sample (mg)

Acceptance criteria: 98.0%–100.5% on the dried basis

### IMPURITIES

#### • ACID-INSOLUBLE SUBSTANCES

Sample: 5.0 g

Analysis: Mix the Sample with 10 mL of water, and add hydrochloric acid, dropwise, with agitation, until it ceases to cause effervescence, then add water to make

the mixture measure 200 mL, and filter. Wash the insoluble residue with water until the last washing shows no chloride, and ignite and weigh the residue.

Acceptance criteria: NMT 0.2%; the weight of the residue does not exceed 10 mg.

#### • ARSENIC, Method I (211)

Sample solution: Slowly dissolve 1.0 g in 15 mL of hydrochloric acid, and dilute with water to 55 mL.

Analysis: Omit the addition of 20 mL of 7 N sulfuric acid specified in *Arsenic* (211), Method I, Procedure.

Acceptance criteria: NMT 3 ppm

- **BARIUM:** A platinum wire, dipped in the filtrate obtained in the test for *Acid-Insoluble Substances* and held in a nonluminous flame, does not impart a green color.

### Delete the following:

#### • HEAVY METALS (231)

Test preparation: Mix 1.0 g with 5 mL of water, slowly add 8 mL of 3 N hydrochloric acid, and evaporate on a steam bath to dryness. Dissolve the residue in 20 mL of water, filter, and add water to the filtrate to make 25 mL.

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

#### • IRON (241)

Sample solution: 40 mg in 5 mL of 2 N hydrochloric acid. Transfer to a beaker with the aid of water, and dilute with water to 10 mL.

Standard solution: Transfer 4.0 mL of the *Standard Iron Solution*, prepared as directed in *Iron* (241), to a beaker, and dilute with water to 10 mL.

#### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Analytical wavelength: 530 nm

Blank: Water

Analysis: Separately to the Sample solution and Standard solution add 2 mL of citric acid solution (1 in 5) and 2 drops of thioglycolic acid, adjust with ammonia TS to a pH of 9.5 ± 0.1, dilute with water to 20 mL, and allow to stand for 5 min. Dilute with water to 50 mL. Concomitantly determine the absorbances of the solutions from the Sample solution and the Standard solution.

Acceptance criteria: NMT 0.1%; the absorbance of the solution from the Sample solution does not exceed that of the Standard solution.

#### • LEAD (251)

Sample solution: 1.0 g in 5 mL of water

Analysis: To the Sample solution slowly add 8 mL of 3 N hydrochloric acid, evaporate on a steam bath to dryness, and dissolve the residue in 5 mL of water.

Acceptance criteria: NMT 3 ppm

#### • LIMIT OF FLUORIDE

[NOTE—Prepare and store all solutions in plastic containers.]

Solution A: 294 mg/mL of sodium citrate dihydrate in water

Sample: 2.0 g

Standard stock solution: 1.11 mg/mL of USP Sodium Fluoride RS in water

Standard solution: Combine 20.0 mL of the Standard stock solution with 50.0 mL of Solution A, and dilute with water to 100.0 mL. [NOTE—Each mL of this solution contains 100 µg of fluoride ion]

Electrode system: Use a fluoride-specific ion-indicating electrode and a silver-silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see pH (791)).

Standard response line: Transfer 50.0 mL of Solution A and 4.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential, in mV. Continue stirring,



and at 5-min intervals add 100, 100, 300, and 500  $\mu\text{L}$  of the *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0  $\mu\text{g/mL}$ ) versus potential, in mV.

**Analysis:** Transfer the *Sample* to a beaker containing a plastic-coated stirring bar, add 20 mL of water and 4.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Solution A* and sufficient water to make 100 mL of test solution. Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line*, determine the concentration,  $C$ , in  $\mu\text{g/mL}$ , of fluoride ion in the *Sample solution*. Calculate the content of fluoride in the specimen taken:

$$\text{Result} = (V \times C)/W$$

$V$  = volume of the *Sample solution* (mL)  
 $C$  = concentration of fluoride in the *Sample solution* ( $\mu\text{g/mL}$ )  
 $W$  = weight of *Sample* (g)

Acceptance criteria: NMT 50 ppm

#### • LIMIT OF MAGNESIUM AND ALKALI SALTS

**Sample solution:** 1.0 g

**Analysis:** Mix the *Sample* with 35 mL of water. Carefully add 3 mL of hydrochloric acid, heat the solution, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well-established. Add immediately to the warm mixture 2 drops of methyl red TS and then 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline. Cool to room temperature, transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Filter, and to 50 mL of the clear filtrate in a platinum dish add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammonium salts. Finally, ignite the residue to constant weight.

Acceptance criteria: NMT 1.0%; the weight of the residue is NMT 5 mg.

#### • MERCURY, Method IIa (261)

**Mercury stock solution and Standard mercury solution:** Proceed as directed in *Mercury* (261).

**Standard solution:** Proceed as directed in *Mercury* (261), except use 3 mL of hydrochloric acid instead of 3 mL of sulfuric acid.

**Sample stock solution:** 4.0 g in a 100-mL beaker, and cautiously dissolve in 14 mL of 6 N hydrochloric acid

**Sample solution:** Proceed as directed in *Mercury* (261) using the *Sample stock solution*, except use 3 mL of hydrochloric acid instead of 3 mL of sulfuric acid.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Proceed as directed in *Mercury* (261).

Acceptance criteria: NMT 0.5 ppm

#### SPECIFIC TESTS

- **LOSS ON DRYING (731):** Dry a sample at 200° for 4 h: it loses NMT 2.0% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

#### • USP REFERENCE STANDARDS (11)

USP Sodium Fluoride RS

### Calcium Carbonate Lozenges

#### DEFINITION

Calcium Carbonate Lozenges contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ).

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** The addition of 6 N hydrochloric acid to a Lozenge produces effervescence, and the resulting solution, after being boiled to expel carbon dioxide and then neutralized with 6 N ammonium hydroxide, meets the requirements of the tests.

#### ASSAY

##### • PROCEDURE

[NOTE—The *Standard solutions* and the *Sample solution* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

**Lanthanum chloride solution:** Transfer 10 g of potassium chloride and 20 g of lanthanum chloride to a 2000-mL volumetric flask. Add 1000 mL of water and 40 mL of hydrochloric acid, mix, and allow to cool. Dilute with water to volume.

**Standard stock solution:** Transfer 250 mg of chelometric standard calcium carbonate, previously dried at 110° for 2 h and then cooled in a desiccator, to a 500-mL volumetric flask. Add 100 mL of water and 12 mL of 1 N hydrochloric acid, swirl to dissolve the calcium carbonate, and allow to cool. Dilute with water to volume. This stock solution contains about 500  $\mu\text{g/mL}$  of calcium carbonate.

**Standard solutions:** To three separate 100-mL volumetric flasks add 2.0, 3.0, and 4.0 mL of the *Standard stock solution*, and dilute each with *Lanthanum chloride solution* to volume. These *Standard solutions* contain 10, 15, and 20  $\mu\text{g/mL}$  of calcium carbonate, respectively.

**Sample stock solution:** Transfer the equivalent to 3000 mg of calcium carbonate, from powdered Lozenges, to a 1000-mL volumetric flask. Add 100 mL of 1 N hydrochloric acid and 300 mL of water, and sonicate to dissolve the powder. Dilute with water to volume.

**Sample solution:** Transfer 5.0 mL of *Sample stock solution* to a 1000-mL volumetric flask, and dilute with *Lanthanum chloride solution* to volume.

#### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Lamp:** Calcium hollow-cathode

**Flame:** Nitrous oxide-acetylene

**Analytical wavelength:** Calcium emission line at 422.7 nm

**Blank:** *Lanthanum chloride solution*

#### Analysis

**Samples:** *Standard solutions*, *Sample solution*, and *Blank*  
 Plot the absorbances of the *Standard solutions* versus their concentrations of calcium carbonate, in  $\mu\text{g/mL}$ , by drawing a straight line best fitting the three plotted points. From the graph determine the concentration,  $C$ , in  $\mu\text{g/mL}$ , of calcium carbonate in the *Sample solution*.

Calculate the percentage of label claim of calcium carbonate ( $\text{CaCO}_3$ ) in the portion of Lozenges taken:

$$\text{Result} = (C/C_u) \times 100$$



- $C$  = measured concentration of calcium carbonate in the *Sample solution* ( $\mu\text{g/mL}$ ), as calculated above
- $C_U$  = nominal concentration of calcium carbonate in the *Sample solution* ( $\mu\text{g/mL}$ )
- Acceptance criteria: 90.0%–110.0%

**OTHER COMPONENTS**• **SODIUM CONTENT** (if so labeled)

[NOTE—The *Standard solutions* and the *Sample solution* may be modified, if necessary, to obtain solutions of suitable concentrations adaptable to the linear or working range of the instrument.]

**Standard stock solution:** Transfer 2.542 g of sodium chloride, previously dried at  $105^\circ$  for 2 h, to a 1000-mL volumetric flask. Dissolve in and dilute with water to volume. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, and dilute with water to volume.

**Standard solutions:** To three separate 100-mL volumetric flasks, add 1.0, 3.0, and 5.0 mL of the *Standard stock solution*, and dilute each with water to volume. These *Standard solutions* contain 1.0, 3.0, and 5.0  $\mu\text{g/mL}$  of sodium, respectively.

**Sample stock solution:** Prepare as directed in the Assay. Pass a portion of it, if necessary, through a filter of 0.5- $\mu\text{m}$  or finer pore size, and use the clear solution.

**Sample solution:** Transfer 10.0 mL of the *Sample stock solution* to a 25-mL volumetric flask, and dilute with water to volume.

**Instrumental conditions**

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Lamp:** Sodium hollow-cathode

**Flame:** Air–acetylene

**Analytical wavelength:** Sodium emission line at 589.6 nm

**Blank:** Water

**Analysis**

**Samples:** *Standard solutions*, *Sample solution*, and *Blank*  
Plot the absorbances of the *Standard solutions* versus their contents of sodium, in  $\mu\text{g/mL}$ , by drawing a straight line best fitting the three plotted points. From the graph determine the quantity,  $C$ , in  $\mu\text{g}$ , of sodium in each mL of the *Sample solution*.

Calculate the percentage of label claim of sodium in the portion of Lozenges taken:

$$\text{Result} = (C/C_U) \times 100$$

- $C$  = measured concentration of sodium in the *Sample solution* ( $\mu\text{g/mL}$ ), as calculated above
- $C_U$  = nominal concentration of sodium in the *Sample solution* ( $\mu\text{g/mL}$ )

Acceptance criteria: NMT 115.0% of the labeled amount

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**SPECIFIC TESTS**• **ACID-NEUTRALIZING CAPACITY** (301)

**Analysis:** The acid consumed by the minimum single dose recommended in the labeling is NLT 5 mEq of acid and NLT the number of mEq calculated by:

$$\text{Result} = (F_c \times C) \times 0.9$$

$F_c$  = theoretical acid-neutralizing capacity of  $\text{CaCO}_3$ , 0.02 mEq

$C$  = quantity of  $\text{CaCO}_3$  in the sample tested (mg), based on the labeled quantity

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

**Calcium Carbonate Oral Suspension****DEFINITION**

Calcium Carbonate Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ).

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** The addition of acetic acid to it produces effervescence (presence of carbonate). The resulting solution, after boiling, meets the requirements.

**ASSAY**• **PROCEDURE**

**Sample solution:** Transfer a portion of Oral Suspension, equivalent to 1 g of calcium carbonate, previously well shaken in its original container, to a beaker with the aid of 25 mL of water. Add 20 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min. Allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to volume. Mix, and filter.

**Blank:** 100 mL of water, 15 mL of 1 N sodium hydroxide, and 5 mL of triethanolamine

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Indicator:** 100 mg of hydroxy naphthol blue

**Endpoint detection:** Visual, change to distinct blue

**Analysis:** Transfer 20.0 mL of the *Sample solution* to a suitable container. Dilute with water to 100 mL. Add 15 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with the *Titrant*.

Calculate the percentage of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) in the sample taken:

$$\text{Result} = [(V_S - V_B) \times M \times F \times 100]/W$$

$V_S$  = volume of the *Titrant* consumed by the *Sample solution* (mL)

$V_B$  = volume of the *Titrant* consumed by the *Blank* (mL)

$M$  = *Titrant* molarity (mmol/mL)

$F$  = equivalency factor, 100.09 mg/mmol

$W$  = nominal amount of calcium carbonate taken for the *Analysis* (mg)

Acceptance criteria: 90.0%–110.0%

**IMPURITIES**• **LIMIT OF FLUORIDE**

[NOTE—Prepare and store all solutions in plastic containers.]

**Solution A:** 294 mg/mL of sodium citrate dihydrate in water

**Standard stock solution:** 1.1 mg/mL of USP Sodium Fluoride RS in water

**Standard solution:** Combine 20.0 mL of the *Standard stock solution* with 50.0 mL of *Solution A*, and dilute with water to 100.0 mL. [NOTE—Each mL of this solution contains 100  $\mu\text{g}$  of fluoride ion.]

**Sample solution:** Transfer a portion of Oral Suspension, equivalent to 2.0 g of calcium carbonate, to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 4.0 mL of hydrochloric acid. Stir until dissolved. Add 50.0 mL of *Solution A* and sufficient water to make 100.0 mL.

**Electrode system:** Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of  $\pm 0.2$  mV (see pH (791)).



**Standard response line:** Transfer 50.0 mL of *Solution A* and 4.0 mL of hydrochloric acid to a beaker. Add water to make 100.0 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, and stir for 15 min. Read the potential, in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500  $\mu$ L of the *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0  $\mu$ g/mL) versus potential, in mV.

**Analysis:** Rinse and dry the electrodes, and insert them into the *Sample solution*. Stir for 5 min, and read the potential, in mV. From the measured potential and the *Standard response line*, determine the concentration,  $C$ , in  $\mu$ g/mL, of fluoride ion in the *Sample solution*. Calculate the content of fluoride in the sample taken:

$$\text{Result} = (V \times C)/W$$

$V$  = volume of the *Sample solution* (mL)  
 $C$  = determined concentration of fluoride in the *Sample solution* ( $\mu$ g/mL)  
 $W$  = nominal weight of calcium carbonate taken (g)

**Acceptance criteria:** 50  $\mu$ g/g, with respect to the labeled amount of calcium carbonate

• **ARSENIC, Method I (211)**

**Test preparation:** Slowly dissolve a portion of Oral Suspension equivalent to 1.0 g of calcium carbonate in 15 mL of hydrochloric acid. Dilute with water to 55 mL.

**Analysis:** Proceed as directed in the chapter, except omit the addition of 20 mL of 7 N sulfuric acid specified under *Procedure*.

**Acceptance criteria:** NMT 3  $\mu$ g/g, with respect to the labeled amount of calcium carbonate

• **LEAD (251)**

**Test preparation:** Mix a portion of Oral Suspension equivalent to 1.0 g of calcium carbonate in 5 mL of water.

**Analysis:** To the *Test preparation* slowly add 8 mL of 3 N hydrochloric acid. Evaporate on a steam bath to dryness, and dissolve the residue in 5 mL of water.

**Acceptance criteria:** NMT 3  $\mu$ g/g, with respect to the labeled amount of calcium carbonate

**Delete the following:**

• **HEAVY METALS (231)**

**Test preparation:** Mix a portion of Oral Suspension equivalent to 1.0 g of calcium carbonate with 5 mL of water. Slowly add 8 mL of 3 N hydrochloric acid, and evaporate on a steam bath to dryness. Dissolve the residue in 20 mL of water. Filter, and add water to the filtrate to make 25 mL.

**Acceptance criteria:** NMT 20  $\mu$ g/g, with respect to the labeled amount of calcium carbonate (Official 1-Jan-2018)

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count is NMT  $10^2$  cfu/mL. It meets the requirements of the tests for absence of *Escherichia coli* and *Pseudomonas aeruginosa*.
- **PH (791):** 7.5–8.7

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.

• **USP REFERENCE STANDARDS (11)**

USP Sodium Fluoride RS

## Calcium Carbonate Tablets

**DEFINITION**

Calcium Carbonate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ). For Tablets labeled for any indication other than, or in addition to, antacid use, the Tablets contain NLT 90.0% and NMT 115.0% of the labeled amount of calcium carbonate.

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** The addition of 6 N acetic acid to the Tablets produces effervescence, and the resulting solution, after being boiled to expel carbon dioxide and neutralized with 6 N ammonium hydroxide, meets the requirements.

**ASSAY**

• **PROCEDURE**

**Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 200 mg of calcium carbonate, to a suitable crucible. Ignite to constant weight. Cool the crucible, add 10 mL of water, and dissolve the residue by adding sufficient 3 N hydrochloric acid, dropwise, to achieve complete solution.

**Blank:** 150 mL of water and 15 mL of 1 N sodium hydroxide

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Indicator:** 300 mg of hydroxy naphthol blue

**Endpoint detection:** Visual, change to distinct blue

**Analysis:** Transfer the *Sample solution* completely to a suitable container, and dilute with water to 150 mL. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue. Titrate with the *Titrant*. Calculate the percentage of calcium carbonate ( $\text{CaCO}_3$ ) in the sample taken:

$$\text{Result} = [(V_S - V_B) \times M \times F \times 100]/W$$

$V_S$  = volume of the *Titrant* consumed by the *Sample solution* (mL)

$V_B$  = volume of the *Titrant* consumed by the *Blank* (mL)

$M$  = *Titrant* molarity (mmol/mL)

$F$  = equivalency factor, 100.09 mg/mmol

$W$  = weight of calcium carbonate taken (mg)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of  $\text{CaCO}_3$ . For Tablets labeled for any indication other than, or in addition to, antacid use, 90.0%–115.0% of the labeled amount of  $\text{CaCO}_3$

**PERFORMANCE TESTS**

• **DISSOLUTION (711)**

[NOTE—For Tablets labeled for any indication other than, or in addition to, antacid use.]

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Lanthanum chloride solution:** 50 mg/mL of lanthanum chloride in 0.1 N hydrochloric acid

**Standard stock solution:** 100  $\mu$ g/mL of calcium in 0.1 N hydrochloric acid

**Standard solutions:** Into separate 100-mL volumetric flasks containing 10.0 mL of *Lanthanum chloride solution* pipet 3-, 4-, 5-, and 6-mL portions of *Standard stock solution* and dilute each with 0.1 N hydrochloric acid to



volume to obtain solutions with calcium concentrations of 3, 4, 5, and 6 µg/mL, respectively.

**Sample solution:** Filter a portion of the solution under test. Pipet a volume of the filtrate, estimated to contain 1 mg of calcium, into a 250-mL volumetric flask. Add 25.0 mL of *Lanthanum chloride solution*, and dilute with 0.1 N hydrochloric acid to volume.

#### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 422.8 nm

**Lamp:** Calcium hollow-cathode

**Flame:** Air-acetylene

**Blank:** *Lanthanum chloride solution* and 0.1 N hydrochloric acid (1:9)

#### Analysis

**Samples:** *Standard solutions* and *Sample solution*

Concomitantly determine the absorbances of the *Standard solutions* and the *Sample solution* against the *Blank*. Construct a standard curve by plotting absorbances versus calcium concentrations of the *Standard solutions*, then from it obtain the concentration,  $C$ , in µg/mL of calcium, of the *Sample solution*.

Calculate the percentage of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) dissolved:

$$\text{Result} = (M_r/A_r) \times (C \times D \times V/L) \times 100$$

$M_r$  = molecular weight of calcium carbonate, 100.09

$A_r$  = atomic weight of calcium, 40.08

$C$  = measured concentration of calcium in the *Sample solution* (mg/mL)

$D$  = dilution factor for the *Sample solution*

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% ( $Q$ ) of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

#### SPECIFIC TESTS

- **ACID-NEUTRALIZING CAPACITY** (301): For Tablets labeled for antacid use

**Analysis:** Proceed as directed in the chapter.

**Acceptance criteria:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated as follows:

$$\text{Result} = (C \times A_{NC}) \times F$$

$C$  = quantity of  $\text{CaCO}_3$  in the sample tested (mg), based on the labeled amount

$A_{NC}$  = theoretical acid-neutralizing capacity of  $\text{CaCO}_3$ , 0.02 mEq/mg

$F$  = acceptance factor for the lower limit of the required acid-neutralizing capacity, 0.9

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label it to indicate whether it is for use as an antacid, or as a dietary supplement, or both.

## Calcium Carbonate and Magnesia Tablets

#### DEFINITION

Calcium Carbonate and Magnesia Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) and NLT 90.0% and NMT

115.0% of the labeled amount of magnesium hydroxide [ $\text{Mg}(\text{OH})_2$ ].

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium** (191): The addition of 3 N hydrochloric acid to the Tablets produces effervescence. The resulting solution, after being boiled to expel carbon dioxide and neutralized with 6 N ammonium hydroxide, meets the requirements of the tests.

- **B. IDENTIFICATION TESTS—GENERAL, Magnesium** (191)

**Sample solution:** Heat 2 Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.

**Acceptance criteria:** The solution meets the requirements.

#### ASSAY

- **CALCIUM CARBONATE**

**Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 400 mg of calcium carbonate, to a beaker with 25 mL of water. Add 40 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to volume, mix, filter, and use the filtrate.

[NOTE—Reserve a portion of it for the test for *Magnesium Hydroxide*.]

**Analysis:** Transfer 20.0 mL of the *Sample solution* to a suitable container, dilute with water to 100 mL, and add 30 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue in color. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of  $\text{CaCO}_3$ .

**Acceptance criteria:** 90.0%–110.0%

- **MAGNESIUM HYDROXIDE**

**Sample solution:** Use the *Sample solution* from the test for *Calcium Carbonate*.

**Analysis:** Transfer a portion of the *Sample solution*, equivalent to 120 mg of calcium carbonate and magnesium hydroxide combined, to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia-ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. The volume, in mL, of 0.05 M edetate disodium consumed, less the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the volume, in mL, of the *Sample solution* taken, represents the volume, in mL, of 0.05 M edetate disodium equivalent to the quantity of magnesium hydroxide present. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of  $\text{Mg}(\text{OH})_2$ .

**Acceptance criteria:** 90.0%–115.0%

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesia

#### SPECIFIC TESTS

- **ACID-NEUTRALIZING CAPACITY** (301)

**Analysis:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated by the formula:

$$\text{Result} = [0.8 \times (F_M \times M)] + [0.9 \times (F_C \times C)]$$

$F_M$  = theoretical acid-neutralizing capacity of  $\text{Mg}(\text{OH})_2$ , 0.0343 mEq

$M$  = quantity of  $\text{Mg}(\text{OH})_2$  in the sample tested (mg), based on the labeled quantity

$F_C$  = theoretical acid-neutralizing capacity of  $\text{CaCO}_3$ , 0.02 mEq



C = quantity of  $\text{CaCO}_3$  in the sample tested (mg), based on the labeled quantity

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

### Calcium Carbonate and Magnesia Chewable Tablets

#### DEFINITION

Calcium Carbonate and Magnesia Chewable Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) and NLT 90.0% and NMT 115.0% of the labeled amount of magnesium hydroxide [ $\text{Mg}(\text{OH})_2$ ].

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** The addition of 3 N hydrochloric acid to the Chewable Tablets produces effervescence. The resulting solution, after being boiled to expel carbon dioxide and neutralized with 6 N ammonium hydroxide, meets the requirements of the tests.
- **B. IDENTIFICATION TESTS—GENERAL, Magnesium (191)**  
**Sample solution:** Heat 2 Chewable Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.  
**Acceptance criteria:** The solution meets the requirements.

#### ASSAY

##### • CALCIUM CARBONATE

**Sample solution:** Finely powder NLT 20 Chewable Tablets. Transfer a portion of the powder, equivalent to 400 mg of calcium carbonate, to a beaker with 25 mL of water. Add 40 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to volume, mix, filter, and use the filtrate. [NOTE—Reserve a portion of it for the test for Magnesium Hydroxide.]

**Analysis:** Transfer 20.0 mL of the *Sample solution* to a suitable container, dilute with water to 100 mL, and add 30 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue in color. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of  $\text{CaCO}_3$ .

**Acceptance criteria:** 90.0%–110.0%

##### • MAGNESIUM HYDROXIDE

**Sample solution:** Use the *Sample solution* from the test for Calcium Carbonate.

**Analysis:** Transfer a portion of the *Sample solution*, equivalent to 120 mg of calcium carbonate and magnesium hydroxide combined, to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. The volume, in mL, of 0.05 M edetate disodium consumed, less the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the volume, in mL, of the *Sample solution* taken, represents the volume, in mL, of 0.05 M edetate disodium equivalent to the quantity of magnesium hydroxide present. Each mL of 0.05 M edetate disodium is equivalent to 2.916 mg of  $\text{Mg}(\text{OH})_2$ .

**Acceptance criteria:** 90.0%–115.0%

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesium

#### SPECIFIC TESTS

##### • ACID-NEUTRALIZING CAPACITY (301)

**Analysis:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated by the formula:

$$\text{Result} = [0.8 \times (F_M \times M)] + [0.9 \times (F_C \times C)]$$

$F_M$  = theoretical acid-neutralizing capacity of  $\text{Mg}(\text{OH})_2$ , 0.0343 mEq

$M$  = quantity of  $\text{Mg}(\text{OH})_2$  in the sample tested (mg), based on the labeled quantity

$F_C$  = theoretical acid-neutralizing capacities of  $\text{CaCO}_3$ , 0.02 mEq

$C$  = quantity of  $\text{CaCO}_3$  in the sample tested (mg), based on the labeled quantity

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Label the Chewable Tablets to indicate that they must be chewed before being swallowed.

### Calcium Carbonate, Magnesia, and Simethicone Chewable Tablets

**Former Title:** Calcium Carbonate, Magnesia, and Simethicone Tablets

» Calcium Carbonate, Magnesia, and Simethicone Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of calcium carbonate ( $\text{CaCO}_3$ ) and magnesium hydroxide [ $\text{Mg}(\text{OH})_2$ ], and an amount of polydimethylsiloxane  $[-(\text{CH}_3)_2\text{SiO}-]_n$  that is not less than 85.0 percent and not more than 115.0 percent of the labeled amount of simethicone.

**Packaging and storage—**Preserve in well-closed containers.

**Labeling—**Label it to indicate that the Chewable Tablets are to be chewed before swallowing. Label the Chewable Tablets to state the sodium content, in mg per Chewable Tablet, if it is greater than 5 mg per Chewable Tablet.

#### USP Reference standards (11)—

USP Polydimethylsiloxane RS

#### Identification—

**A: Infrared Absorption (197S)—**

**Solution—**Using Chewable Tablets, proceed to obtain IR absorption spectra as directed in the *Assay for polydimethylsiloxane under Alumina, Magnesia, and Simethicone Chewable Tablets*.

**B:** The addition of 1 N hydrochloric acid to a Chewable Tablet produces effervescence, and the resulting solution, after having been filtered, meets the requirements of the tests for Calcium (191).

**C:** Heat 2 Chewable Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 minutes. Filter this solution, and to the filtrate add 2 mL of 1 N hydrochloric acid: this solution meets the requirements of the tests for Magnesium (191).



**Uniformity of dosage units** (905): meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesium hydroxide.

**Acid-neutralizing capacity** (301)—Not less than 5 mEq of acid is consumed by the minimum single dose recommended in the labeling.

**Content of sodium** (if so labeled)—

*Lanthanum chloride solution*—Prepare as directed in the *Assay for calcium carbonate and magnesium hydroxide*.

*Dilute hydrochloric acid*—Prepare as directed in the *Assay for polydimethylsiloxane*.

*Standard solution*—Transfer 2.542 g of sodium chloride, previously dried at 105° for 2 hours, to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 4.0 mL of this solution to a second 100-mL volumetric flask containing 6.0 mL of *Dilute hydrochloric acid* and 2.0 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix. This solution contains 2.0 µg of sodium (Na) per mL.

*Test solution*—Transfer 3.0 mL of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* to a 50-mL volumetric flask containing 1.0 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix.

*Blank solution*—Transfer 15.0 mL of *Dilute hydrochloric acid* and 5.0 mL of *Lanthanum chloride solution* to a 250-mL volumetric flask, dilute with water to volume, and mix.

*Procedure*—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the sodium emission line at 589.0 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a sodium hollow-cathode lamp and an air-acetylene flame, using the *Blank solution* as the blank. Calculate the mg of sodium (Na) in each Chewable Tablet taken by the formula:

$$(5C/6)(A/W)(A_U / A_S)$$

in which C is the concentration, in µg per mL, of sodium in the *Standard solution*; A is the average weight, in mg, of each Chewable Tablet; W is the weight, in mg, of the portion of Chewable Tablets from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* used to prepare the *Test solution*; and  $A_U$  and  $A_S$  are the absorbances of the *Test solution* and the *Standard solution*, respectively. Each Chewable Tablet contains not more than the number of mg of sodium stated on the label.

#### **Assay for polydimethylsiloxane—**

*Saccharin solution*—Prepare a solution of saccharin in 4-methyl-2-pentanone containing 12.5 mg per mL.

*Dilute hydrochloric acid*—Mix 200 mL of hydrochloric acid with sufficient water to make 1000 mL.

*Standard preparation*—Dissolve a suitable quantity of USP Polydimethylsiloxane RS in 4-methyl-2-pentanone to obtain a stock solution having a known concentration of about 1 mg per mL. On the day of use, transfer 20.0 mL of this solution and 5.0 mL of *Saccharin solution* to a 250-mL volumetric flask, dilute with 4-methyl-2-pentanone to volume, and mix. This solution contains about 0.08 mg of USP Polydimethylsiloxane RS per mL.

*Assay preparation*—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 20 mg of polydimethylsiloxane, to a 125-mL separator. Cautiously add 50.0 mL of *Dilute hydrochloric acid*, and swirl until the reaction subsides. Insert the stopper, and mix. Carefully release the pressure, add 50.0 mL of 4-methyl-2-pentanone, and mix for 10 minutes. Allow the layers to separate, and drain the aqueous layer into a suitable stoppered container. [NOTE—Retain this aqueous layer for use in preparing the *Assay preparation* in the *Assay for calcium carbonate and*

*magnesium hydroxide* and for the preparation of the *Test solution* in the test for *Content of sodium*.] Filter the organic layer through a filter containing 50 g of anhydrous sodium sulfate. Transfer 10.0 mL of the filtrate to a 50-mL volumetric flask, add 1.0 mL of *Saccharin solution*, dilute with methyl isobutyl ketone to volume, and mix.

*Blank solution*—Transfer 1.0 mL of *Saccharin solution* to a 50-mL volumetric flask, dilute with 4-methyl-2-pentanone to volume, and mix.

*Procedure*—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the silicon emission line at 251.6 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a silicon hollow-cathode lamp and a nitrous oxide-acetylene flame, using the *Blank solution* as the blank. Calculate the quantity, in mg, of polydimethylsiloxane in each Chewable Tablet taken by the formula:

$$250C(A/W)(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Polydimethylsiloxane RS in the *Standard preparation*; A is the average weight, in mg, of each Chewable Tablet; W is the weight, in mg, of the portion of Chewable Tablets taken to prepare the *Assay preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

#### **Assay for calcium carbonate and magnesium hydroxide—**

*Lanthanum chloride solution*—Transfer 26.8 g of lanthanum chloride to a 200-mL volumetric flask, add 100 mL of water, and carefully add 50 mL of hydrochloric acid. Mix, and allow to cool. Dilute with water to volume, and mix.

*Dilute hydrochloric acid*—Prepare as directed in the *Assay for polydimethylsiloxane*.

*Calcium stock standard solution*—Transfer 499.5 mg of primary standard calcium carbonate to a 200-mL volumetric flask, and add 10 mL of water. Carefully add 5 mL of *Dilute hydrochloric acid*, and swirl to dissolve the calcium carbonate. Dilute with water to volume, and mix. This solution contains 1000 µg of calcium (Ca) per mL.

*Magnesium stock standard solution*—Transfer 1.000 g of magnesium metal to a 1000-mL volumetric flask containing 10 mL of water, slowly add 10 mL of hydrochloric acid, and swirl to dissolve the metal. Dilute with water to volume, and mix. This solution contains 1000 µg of magnesium (Mg) per mL.

*Calcium and magnesium standard preparation*—To a 250-mL volumetric flask add 10.0 mL of *Calcium stock standard solution* and 5.0 mL of *Magnesium stock standard solution*, dilute with water to volume, and mix. This solution contains 40 µg of calcium (Ca) and 20 µg of magnesium (Mg) per mL. On the day of use, transfer 4.0 mL of this solution to a 100-mL volumetric flask containing 2.0 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix. This solution contains 1.6 µg of calcium (Ca) and 0.8 µg of magnesium (Mg) per mL.

*Assay preparation*—Transfer an accurately measured volume of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane*, equivalent to about 28 mg of calcium carbonate, to a 200-mL volumetric flask, dilute with water to volume, and mix. Transfer 3.0 mL of this solution to a 100-mL volumetric flask containing 2.0 mL of *Lanthanum chloride solution*, dilute with water to volume, and mix.

*Blank solution*—Transfer 5.0 mL of *Lanthanum chloride solution* to a 250-mL volumetric flask, dilute with water to volume, and mix.

*Procedure for calcium carbonate*—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the calcium emission line at 422.7 nm, with a suitable atomic absorption spectrophotometer (see



Atomic Absorption Spectroscopy (852)) equipped with a calcium hollow-cathode lamp and a nitrous oxide-acetylene flame, using the *Blank solution* as the blank. Calculate the quantity, in mg, of calcium carbonate ( $\text{CaCO}_3$ ) in each Chewable Tablet taken by the formula:

$$(100.09/40.08)(1000C/3V)(A/W)(A_U / A_S)$$

in which 100.09 is the molecular weight of calcium carbonate; 40.08 is the atomic weight of calcium; C is the concentration, in  $\mu\text{g}$  per mL, of calcium in the *Standard preparation*; V is the volume, in mL, of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* used to prepare the *Assay preparation*; A is the average weight, in mg, of each Chewable Tablet; W is the weight, in mg, of the portion of Chewable Tablets taken to prepare the *Assay preparation* in the *Assay for polydimethylsiloxane*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

*Procedure for magnesium hydroxide*—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the magnesium emission line at 285.2 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a magnesium hollow-cathode lamp and a nitrous oxide-acetylene flame, using the *Blank solution* as the blank. Calculate the quantity, in mg, of magnesium hydroxide [ $\text{Mg}(\text{OH})_2$ ] in each Chewable Tablet taken by the formula:

$$(58.34/24.305)(1000C/3V)(A/W)(A_U / A_S)$$

in which 58.34 is the molecular weight of magnesium hydroxide; 24.305 is the atomic weight of magnesium; C is the concentration, in  $\mu\text{g}$  per mL, of magnesium in the *Standard preparation*; V is the volume, in mL, of the aqueous layer retained from the preparation of the *Assay preparation* in the *Assay for polydimethylsiloxane* used to prepare the *Assay preparation*; A is the average weight, in mg, of each Chewable Tablet taken; W is the weight, in mg, of the portion of Chewable Tablets taken to prepare the *Assay preparation* in the *Assay for polydimethylsiloxane*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Calcium and Magnesium Carbonates Oral Suspension

### DEFINITION

Calcium and Magnesium Carbonates Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) and NLT 85.0% and NMT 115.0% of the labeled amount of magnesium carbonate ( $\text{MgCO}_3$ ).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** The addition of 3 N hydrochloric acid to a quantity of Oral Suspension, equivalent to 500 mg of calcium carbonate, produces effervescence, and the resulting solution, after having been filtered, meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Magnesium (191)**  
**Sample solution:** Heat a quantity of Oral Suspension, equivalent to 800 mg of magnesium carbonate, with 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • CALCIUM CARBONATE

**Sample solution:** Transfer a portion of Oral Suspension equivalent to 400 mg of calcium carbonate, previously well shaken in its original container and free of air bubbles, to a beaker, with the aid of 20 mL of water, and add 10 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, transfer with the aid of water to a 100-mL volumetric flask, dilute with water to volume, filter and use the filtrate. [NOTE—Reserve a portion of the filtrate for the *Sample solution* in the *Magnesium Carbonate* test.]

**Analysis:** Transfer 20.0 mL of *Sample solution* to a suitable container. Dilute with water to 100 mL, and add 15 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of  $\text{CaCO}_3$ .

**Acceptance criteria:** 90.0%–110.0%

#### • MAGNESIUM CARBONATE

**Sample solution:** Use a portion of the filtrate from the *Sample solution* in the *Calcium Carbonate* test.

**Analysis:** Transfer the *Sample solution*, equivalent to 120 mg of calcium carbonate and magnesium carbonate combined, to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia-ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. From the volume of 0.05 M edetate disodium consumed, subtract the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the portion of the *Sample solution* taken. The difference is the volume of 0.05 M edetate disodium equivalent to the quantity of magnesium carbonate present. Each mL of 0.05 M edetate disodium is equivalent to 4.216 mg of  $\text{MgCO}_3$ .

**Acceptance criteria:** 85.0%–115.0%

### PERFORMANCE TESTS

- **DELIVERABLE VOLUME (698):** Meets the requirements

### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count is NMT 100 cfu/mL, and it meets the requirements of the tests for absence of *Escherichia coli* and *Pseudomonas aeruginosa*.
- **PH (791):** 7.0–8.6
- **ACID-NEUTRALIZING CAPACITY (301):** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated:

$$\text{Result} = [(F_M \times M) \times 0.8] + [(F_C \times C) \times 0.9]$$

- $F_M$  = theoretical acid-neutralizing capacity of  $\text{MgCO}_3$ , 0.024 mEq
- $M$  = quantity of  $\text{MgCO}_3$  in the specimen tested, based on the labeled quantity (mg)
- $F_C$  = theoretical acid-neutralizing capacity of  $\text{CaCO}_3$ , 0.02 mEq
- $C$  = quantity of  $\text{CaCO}_3$  in the specimen tested, based on the labeled quantity (mg)

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid freezing.



## Calcium and Magnesium Carbonates Tablets

### DEFINITION

Calcium and Magnesium Carbonates Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of calcium carbonate ( $\text{CaCO}_3$ ) and NLT 85.0% and NMT 115.0% of the labeled amount of magnesium carbonate ( $\text{MgCO}_3$ ).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** The addition of 1 N hydrochloric acid to 1 Tablet produces effervescence, and the resulting solution, after having been filtered, meets the requirements of the tests.
- **B. IDENTIFICATION TESTS—GENERAL, Magnesium (191)**  
**Sample solution:** Heat 2 Tablets in 20 mL of 1 N sulfuric acid. Cool, add 20 mL of alcohol, mix, and allow to stand for 30 min. Filter this solution, and add 2 mL of 1 N hydrochloric acid to the filtrate.  
**Acceptance criteria:** Meet the requirements

### ASSAY

#### • CALCIUM CARBONATE

**Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 400 mg of calcium carbonate, to a beaker with the aid of 25 mL of water, and add 10 mL of 1 N hydrochloric acid. Heat on a steam bath for 30 min, allow to cool, and transfer to a 100-mL volumetric flask with the aid of water. Dilute with water to volume, mix, filter, and use the filtrate. [NOTE—Reserve a portion of the filtrate for the *Sample solution* in the *Magnesium Carbonate* test.]

**Analysis:** Transfer 20.0 mL of *Sample solution* to a suitable container. Dilute with water to 100 mL, and add 15 mL of 1 N sodium hydroxide, 5 mL of triethanolamine, and 100 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg of  $\text{CaCO}_3$ .

**Acceptance criteria:** 90.0%–110.0%

#### • MAGNESIUM CARBONATE

**Sample solution:** Use a portion of the filtrate from the *Sample solution* in the *Calcium Carbonate* test.

**Analysis:** Transfer the *Sample solution* equivalent to 120 mg of calcium carbonate and magnesium carbonate combined to a suitable container. Dilute with water to 100 mL, and add 10 mL of ammonia–ammonium chloride buffer TS, 5 mL of triethanolamine, and 0.3 mL of eriochrome black TS. Titrate with 0.05 M edetate disodium VS to a blue endpoint. From the volume of 0.05 M edetate disodium consumed, subtract the volume of 0.05 M edetate disodium corresponding to the content of calcium carbonate in the portion of the *Sample solution* taken. The difference is the volume of 0.05 M edetate disodium equivalent to the quantity of magnesium carbonate present. Each mL of 0.05 M edetate disodium is equivalent to 4.216 mg of  $\text{MgCO}_3$ .

**Acceptance criteria:** 85.0%–115.0%

### PERFORMANCE TESTS

#### • DISINTEGRATION (701)

**Time:** NMT 10 min, except that where Tablets are labeled as gelatin-coated, the time is NMT 30 min, simulated gastric fluid TS being substituted for water in the test

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Weight Variation* with respect to calcium carbonate and to magnesium carbonate

### SPECIFIC TESTS

#### • ACID-NEUTRALIZING CAPACITY (301)

**Analysis:** NLT 5 mEq of acid is consumed by the minimum single dose recommended in the labeling, and NLT the number of mEq calculated:

$$\text{Result} = [(F_M \times M) \times 0.8] + [(F_C \times C) \times 0.9]$$

$F_M$  = theoretical acid-neutralizing capacity of  $\text{MgCO}_3$ , 0.024 mEq

$M$  = quantity of  $\text{MgCO}_3$  in the specimen tested, based on the labeled quantity (mg)

$F_C$  = theoretical acid-neutralizing capacity of  $\text{CaCO}_3$ , 0.02 mEq

$C$  = quantity of  $\text{CaCO}_3$  in the specimen tested, based on the labeled quantity (mg)

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** Tablets that are gelatin-coated are so labeled.

## Calcium Chloride

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  147.01  
 Calcium chloride dihydrate [10035-04-8].

$\text{CaCl}_2$  110.98  
 Anhydrous [10043-52-4].

### DEFINITION

Calcium Chloride contains an amount of  $\text{CaCl}_2$  equivalent to NLT 99.0% and NMT 107.0% of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191)**  
**Sample solution:** 100 mg/mL  
**Acceptance criteria:** Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Chloride (191)**  
**Sample solution:** 100 mg/mL  
**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Sample solution:** Dissolve 1 g of Calcium Chloride in a mixture of water and 3 N hydrochloric acid (100:5). Transfer the solution to a 250-mL volumetric flask, and dilute with water to volume.

**Analysis:** Pipet 50 mL of the *Sample solution* into a suitable container, and add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue. Titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 7.351 mg of calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ).

**Acceptance criteria:** 99.0%–107.0% of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

### IMPURITIES

#### • ALUMINUM (206)

[NOTE—Perform if labeled as intended for use in hemodialysis.]



Test preparation: Use a 2.0-g sample.  
Acceptance criteria: NMT 1 ppm

#### Delete the following:

- **HEAVY METALS** (231)

Sample solution: 80 mg/mL

Acceptance criteria: NMT 10 ppm (Official 1-Jan-2018)

- **IRON, ALUMINUM, AND PHOSPHATE**

Sample solution: 50 mg/mL

**Analysis:** Add 2 drops of 3 N hydrochloric acid and 1 drop of phenolphthalein TS to the *Sample solution*. Then add ammonium chloride–ammonium hydroxide TS, dropwise, until the solution is faintly pink. Add 2 drops in excess, and heat the liquid to boiling.

**Acceptance criteria:** No turbidity or precipitate is produced.

- **LIMIT OF MAGNESIUM AND ALKALI SALTS**

Sample solution: 20 mg/mL

**Analysis:** To 50 mL of *Sample solution* add 500 mg of ammonium chloride. Heat the solution, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Add immediately to the warm mixture 2 drops of methyl red TS and then 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline. Cool to room temperature. Transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Filter, and to 50 mL of the clear filtrate in a platinum dish, add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammonium salts. Finally, ignite the residue to constant weight.

**Acceptance criteria:** The weight of the residue is NMT 5 mg (1.0%).

#### SPECIFIC TESTS

- **pH** (791)

Sample solution: 50 mg/mL

Acceptance criteria: 4.5–9.2

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where Calcium Chloride is intended for use in hemodialysis, it is so labeled.

### Calcium Chloride Injection

» Calcium Chloride Injection is a sterile solution of Calcium Chloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .

**Packaging and storage—**Preserve in single-dose containers, preferably of Type I glass.

**Labeling—**The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards** (11)—

USP Endotoxin RS

**Identification—**It responds to the tests for *Calcium* and *Chloride* (191).

**Bacterial Endotoxins Test** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of calcium chloride.

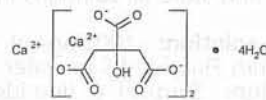
**pH** (791): between 5.5 and 7.5 in the undiluted Injection, except where the concentration is greater than 1 in 20, in which case this range applies to the Injection diluted with water to yield a concentration of 1 in 20.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements—**It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay—**Transfer an accurately measured volume of Injection, equivalent to about 1 g of calcium chloride, to a 250-mL volumetric flask, add 5 mL of 3 N hydrochloric acid, dilute with water to volume, and mix. Pipet 50 mL of the resulting solution into a suitable container, add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue, and titrate with 0.05 M edetate disodium VS until the solution is deep blue. Each mL of 0.05 M edetate disodium is equivalent to 7.351 mg of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .

### Calcium Citrate



$\text{C}_{12}\text{H}_{10}\text{Ca}_3\text{O}_{14} \cdot 4\text{H}_2\text{O}$  570.49

1,2,3-Propanetricarboxylic acid, 2-hydroxy-, calcium salt (2:3), tetrahydrate;

Calcium citrate (3:2), tetrahydrate [5785-44-4].

#### DEFINITION

Calcium Citrate contains four molecules of water of hydration. When dried at 150° to constant weight, it contains NLT 97.5% and NMT 100.5% of  $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ .

#### IDENTIFICATION

- **A.**

**Analysis:** Dissolve 0.5 g in a mixture of 10 mL of water and 2.5 mL of 2 N nitric acid. Add 1 mL of mercuric sulfate TS, heat to boiling, and add 1 mL of potassium permanganate TS.

**Acceptance criteria:** A white precipitate is formed.

- **B.**

**Sample:** 0.5 g of Calcium Citrate

**Analysis:** Ignite completely the *Sample* at as low a temperature as possible, cool, and dissolve the residue in dilute glacial acetic acid (1:10). Filter, and add 10 mL of ammonium oxalate TS to the filtrate.

**Acceptance criteria:** A voluminous white precipitate that is soluble in hydrochloric acid is formed.

#### ASSAY

- **PROCEDURE**

**Sample solution:** Dissolve 350 mg of Calcium Citrate, previously dried at 150° to constant weight, in 12 mL of 0.5 M hydrochloric acid, and dilute with water to about 100 mL.

**Analysis:** While stirring the *Sample solution*, add 30 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 8.307 mg of calcium citrate ( $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2$ ).



Acceptance criteria: 97.5%–100.5% on the dried basis

### IMPURITIES

#### • ARSENIC, Method I (211)

**Test preparation:** Dissolve 1 g of Calcium Citrate in 5 mL of 3 N hydrochloric acid, and dilute with water to 35 mL.

Acceptance criteria: NMT 3 ppm

Delete the following:

#### • HEAVY METALS, Method I (231)

**Test preparation:** Dissolve 1 g of Calcium Citrate in a mixture of hydrochloric acid and water (2:20). Add 1.5 mL of ammonium hydroxide, and dilute with water to 25 mL.

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

#### • LEAD (251)

**Test preparation:** Dissolve 0.5 g of Calcium Citrate in 20 mL of 3 N hydrochloric acid. Evaporate this solution on a steam bath to 10 mL, dilute with water to 20 mL, and cool. Use 5 mL of *Diluted Standard Lead Solution* (5 µg of Pb) for the test.

Acceptance criteria: NMT 10 ppm

#### • LIMIT OF FLUORIDE

[NOTE—Prepare and store all solutions in plastic containers.]

**Standard stock solution:** 1000 µg/mL of fluoride ion from USP Sodium Fluoride RS in water

**Standard solution:** 5 µg/mL of fluoride ion from *Standard stock solution*. [NOTE—Prepare on the day of use.]

**Linearity solution A:** Transfer 1.0 mL of the *Standard solution* to a 250-mL plastic beaker. Add 50 mL of water, 5 mL of 1 N hydrochloric acid, 10 mL of 1.0 M sodium citrate, and 10 mL of 0.2 M edetate disodium. If necessary, adjust with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 5.5. Transfer to a 100-mL volumetric flask, and dilute with water to volume. This solution contains 0.05 µg/mL of fluoride.

**Linearity solution B:** Transfer 5.0 mL of the *Standard solution* to a 250-mL plastic beaker, and proceed as directed for *Linearity solution A* beginning with "Add 50 mL of water". This solution contains 0.25 µg/mL of fluoride.

**Linearity solution C:** Transfer 10.0 mL of the *Standard solution* to a 250-mL plastic beaker, and proceed as directed for *Linearity solution A* beginning with "Add 50 mL of water". This solution contains 0.50 µg/mL of fluoride.

**Sample solution:** Transfer 1.0 g of Calcium Citrate to a 100-mL beaker. Add 10 mL of water and, while stirring, 10 mL of 1 N hydrochloric acid. When dissolved, boil rapidly for 1 min, transfer the solution to a 250-mL plastic beaker, and cool in ice water. Add 15 mL of 1.0 M sodium citrate and 10 mL of 0.2 M edetate disodium, and adjust with 1 N sodium hydroxide or 1 N hydrochloric acid to a pH of 5.5. Transfer this solution to a 100-mL volumetric flask, and dilute with water to volume.

**Electrode system:** Use a fluoride-specific, ion-indicating electrode and a silver-silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of ±0.2 mV (see pH (791)).

#### Analysis

**Samples:** *Linearity solution A*, *Linearity solution B*, *Linearity solution C*, and *Sample solution*

Transfer 50 mL of each *Linearity solution A*, *Linearity solution B*, and *Linearity solution C* to separate 250-mL plastic beakers, and measure the potential of each solution with the *Electrode system*. Between each reading wash the electrodes with water, and absorb any residual water by blotting the electrodes dry. Plot the logarithms of the fluoride concentrations (0.05, 0.25,

and 0.50 µg/mL, respectively) versus potential to obtain a Standard response line.

Transfer 50 mL of the *Sample solution* to a 250-mL plastic beaker, and measure the potential with the *Electrode system*. From the measured potential and the Standard response line determine the concentration, *C*, in µg/mL, of fluoride ion in the *Sample solution*. Calculate the percentage of fluoride in the specimen taken by multiplying *C* by 0.01.

Acceptance criteria: NMT 0.003%

#### • LIMIT OF ACID-INSOLUBLE SUBSTANCES

**Sample solution:** Dissolve 5 g of Calcium Citrate by heating with a mixture of hydrochloric acid and water (10:50) for 30 min.

**Analysis:** Filter, wash, and dry at 105° for 2 h the residue so obtained.

Acceptance criteria: The weight of the residue is NMT 10 mg (0.2%).

### SPECIFIC TESTS

• **LOSS ON DRYING (731):** Dry a sample at 150° for 4 h; it loses from 10.0% to 13.3% of its weight.

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**  
USP Sodium Fluoride RS

## Calcium Glubionate Syrup

### DEFINITION

Calcium Glubionate Syrup is a solution containing equimolar amounts of Calcium Gluconate and Calcium Lactobionate or with Calcium Lactobionate predominating. It contains NLT 95.0% and NMT 105.0% of the labeled amount of calcium (Ca).

### IDENTIFICATION

#### • A. IDENTIFICATION TESTS—GENERAL, Calcium (191)

**Sample solution:** Syrup in water (1 in 10)

Acceptance criteria: Meets the requirements

#### • B. THIN-LAYER CHROMATOGRAPHY

**Standard solution:** 2 mg/mL of calcium gluconate and 4 mg/mL of calcium lactobionate in water

**Sample solution:** Equivalent to 0.4 mg/mL of calcium, from Syrup in water

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 5 µL

**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber and dry at 100° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for 10 min.

Acceptance criteria: The two principal spots of the *Sample solution* correspond in color, size, and *R<sub>f</sub>* value to those obtained from the *Standard solution*. [NOTE—Sucrose, if present in the *Sample solution*, appears in the chromatogram as a spot between the two principal spots.]



**ASSAY****• PROCEDURE**

**Sample solution:** Transfer a portion of Syrup equivalent to 70 mg of Ca into a suitable beaker. Add 2 mL of 3 N hydrochloric acid, and dilute with water to 150 mL.

**Analysis:** While stirring the *Sample solution* with a magnetic stirrer, add 20 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca).

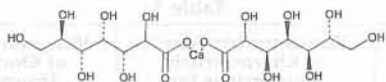
Acceptance criteria: 95.0%–105.0%

**SPECIFIC TESTS**

**• PH (791):** 3.4–4.5

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in tight containers, at a temperature not exceeding 30°, and avoid freezing.

**Calcium Gluceptate**

$C_{14}H_{26}CaO_{16}$  (anhydrous) 490.42  
Glucoheptonic acid, calcium salt (2:1);  
Calcium glucoheptonate (1:2) [29039-00-7].

**DEFINITION**

Calcium Gluceptate is anhydrous or contains varying amounts of water of hydration. It consists of the calcium salt of the alpha epimer of glucoheptonic acid or of a mixture of the alpha and beta epimers of glucoheptonic acid. It contains NLT 95.0% and NMT 102.0% of calcium gluceptate ( $C_{14}H_{26}CaO_{16}$ ), calculated on the dried basis.

**IDENTIFICATION**

- A. INFRARED ABSORPTION (197K)**
- B. IDENTIFICATION TESTS—GENERAL, Calcium (191):** A 20-mg/mL solution meets the requirements.

**ASSAY****• PROCEDURE**

**Sample:** 800 mg of Calcium Gluceptate

**Blank:** 150 mL of water containing 2 mL of 3 N hydrochloric acid

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* with 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, preferably with a magnetic stirrer, add 25 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.

Calculate the percentage of calcium gluceptate ( $C_{14}H_{26}CaO_{16}$ ) in the *Sample* taken:

$$\text{Result} = \left\{ \frac{(V_s - V_b) \times M \times F}{W} \right\} \times 100$$

- $V_s$  = *Titrant* volume consumed by the *Sample* (mL)
- $V_b$  = *Titrant* volume consumed by the *Blank* (mL)
- $M$  = actual molarity of the *Titrant* (mM/mL)
- $F$  = equivalency factor, 490.4 mg/mM
- $W$  = *Sample* weight (mg)

Acceptance criteria: 95.0%–102.0% on the dried basis

**IMPURITIES**

**• CHLORIDE AND SULFATE, Chloride (221)**

**Standard:** 1.0 mL of 0.020 N hydrochloric acid

**Sample:** 1.0 g

Acceptance criteria: NMT 0.07%

**• CHLORIDE AND SULFATE, Sulfate (221)**

**Standard:** 1.0 mL of 0.020 N sulfuric acid

**Sample:** 2.0 g

Acceptance criteria: NMT 0.05%

**Delete the following:**

**• HEAVY METALS (231)**

**Test preparation:** Dissolve 1 g in 25 mL of water.

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

**• REDUCING SUGARS**

**Sample:** 0.50 g

**Analysis:** Dissolve the *Sample* in 10 mL of hot water, add 2 mL of 3 N hydrochloric acid, boil for about 2 min, and cool. Add 5 mL of sodium carbonate TS, allow to stand for 5 min, dilute with water to 20 mL, and filter. Add 5 mL of the clear filtrate to 2 mL of alkaline cupric tartrate TS, and boil for 1 min.

Acceptance criteria: No red precipitate is formed immediately.

**SPECIFIC TESTS**

**• PH (791)**

**Sample solution:** 100 mg/mL

Acceptance criteria: 6.0–8.0

**• LOSS ON DRYING (731)**

(See *Thermal Analysis* (891).)

[NOTE—The quantity taken for the determination may be adjusted, if necessary, for instrument sensitivity. Weight loss occurring at temperatures above about 160°, indicative of decomposition, is not to be interpreted as *Loss on Drying*.]

**Sample:** 10–25 mg

**Analysis:** Determine the percentage of volatile substances by thermogravimetric analysis on an appropriately calibrated instrument. Heat the specimen under test at a rate of 5°/min in an atmosphere of nitrogen, at a flow rate of 40 mL/min. Record the thermogram to 150°.

Acceptance criteria: See *Table 1*.

**Table 1**

Form	Loss on Drying (%)
Anhydrous	NMT 1.0
2H <sub>2</sub> O (dihydrate)	NMT 6.9
3 1/2 H <sub>2</sub> O	NMT 11.4

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers.
- LABELING:** Label to indicate whether hydrous or anhydrous; if hydrous, label to indicate also the degree of hydration.
- USP REFERENCE STANDARDS (11)**  
USP Calcium Gluceptate RS

**Calcium Gluceptate Injection**

» Calcium Gluceptate Injection is a sterile solution of Calcium Gluceptate in Water for Injection. It contains not less than 95.0 percent and not



more than 105.0 percent of the labeled amount of calcium (Ca).

**Packaging and storage**—Preserve in tight, single-dose containers, preferably of Type I or Type II glass.

**Labeling**—The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards (11)**—

USP Calcium Gluceptate RS

USP Endotoxin RS

**Identification**—

**A: Infrared Absorption (197K)**—Prepare the test specimen as follows. Transfer 5 mL of Injection to a separator, add 10 mL of chloroform, shake, and allow the layers to separate. Draw off and discard the chloroform layer, and repeat the extraction with a second 10-mL portion of chloroform. Drain the water layer into a small beaker, evaporate to dryness, and dry in vacuum at 60° for 16 hours.

**B:** A dilution of the Injection with water (1 in 5) responds to the tests for Calcium (191).

**Bacterial Endotoxins Test (85)**—It contains not more than 0.32 USP Endotoxin Unit per mg of calcium gluceptate.

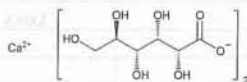
**pH (791):** between 5.6 and 7.0.

**Particulate Matter in Injections (788):** meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products (1)*.

**Assay**—To an accurately measured volume of Injection, equivalent to about 45 mg of calcium, add 2 mL of 3 N hydrochloric acid and 148 mL of water. While stirring, preferably with a magnetic stirrer, add about 15 mL of 0.05 M edetate disodium VS from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca).

## Calcium Gluconate



$C_{12}H_{22}CaO_{14}$  430.37

$C_{12}H_{22}CaO_{14} \cdot H_2O$  448.39

D-Gluconic acid, calcium salt (2:1);

Calcium D-gluconate (1:2) [299-28-5].

Monohydrate [18016-24-5].

**DEFINITION**

Calcium Gluconate is anhydrous or contains one molecule of water of hydration. The anhydrous form contains NLT 98.0% and NMT 102.0% of calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ), calculated on the dried basis. The monohydrate form contains NLT 99.0% and NMT 101.0% of calcium gluconate monohydrate ( $C_{12}H_{22}CaO_{14} \cdot H_2O$ ) where labeled as intended for use in preparing injectable dosage forms, and NLT 98.5% and NMT 102.0% of calcium gluconate monohydrate ( $C_{12}H_{22}CaO_{14} \cdot H_2O$ ) where labeled as not intended for use in preparing injectable dosage forms.

**IDENTIFICATION**

• **A. IDENTIFICATION TESTS—GENERAL (191), Calcium**

Sample solution: 20 mg/mL

Acceptance criteria: Meets the requirements

• **B. INFRARED ABSORPTION**

**Standard preparation:** Prepare USP Calcium Gluconate Anhydrous RS or USP Calcium Gluconate Monohydrate RS in the form of a potassium bromide pellet.

**Sample preparation:** Prepare calcium gluconate anhydrous or calcium gluconate monohydrate in the form of a potassium bromide pellet.

**Analysis**

**Samples:** *Standard preparation* and *Sample preparation* Record the spectra over the range from about 2.6 to 15  $\mu$ m (3800–650  $cm^{-1}$ ).

**Acceptance criteria:** The spectrum of Calcium Gluconate labeled as anhydrous exhibits the differential maxima at the wavenumbers in Table 1 that are consistent with those of the spectrum of USP Calcium Gluconate Anhydrous RS. The spectrum of Calcium Gluconate labeled as monohydrate exhibits the differential maxima at the wavenumbers in Table 1 that are consistent with those of the spectrum of USP Calcium Gluconate Monohydrate RS.

Table 1

Spectral Region	Wavenumber ( $cm^{-1}$ ) of Characteristic Absorptions for Anhydrous	Wavenumber ( $cm^{-1}$ ) of Characteristic Absorptions for Monohydrate
O-H stretching	No sharp band at about 3485, only broad absorption	At about 3485 (medium sharp over broad absorption)
C=O stretching	1618 (strong)	1595 (strong)
	1329 (medium)	Not observed
	Not observed	1305 (medium)
	1263–1250 (strong, two fused bands)	Not observed
	Not observed	1236 (medium)
	1007 (medium)	Not observed
	Not observed	1045 (medium)
	948 (duplet, medium)	Not observed
	Not observed	972 (weak)
	865 (weak)	Not observed
	Not observed	878 (weak)
Fingerprint	766 (medium)	Not observed

**ASSAY**

• **PROCEDURE**

**Sample:** 800 mg of calcium gluconate anhydrous or calcium gluconate monohydrate

**Blank:** 150 mL of water containing 2 mL of 3 N hydrochloric acid

**Titrimetric system**

(See *Titrimetry (541)*.)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring, add 30 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.



Calculate the percentage of calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ) or calcium gluconate monohydrate ( $C_{12}H_{22}CaO_{14} \cdot H_2O$ ) in the *Sample* taken:

$$\text{Result (anhydrous form)} = \left\{ \frac{(V_S - V_B) \times M \times F}{W} \right\} \times 100$$

$$\text{Result (monohydrate form)} = \left\{ \frac{(V_S - V_B) \times M \times F}{W} \right\} \times \left( \frac{M_{12}}{M_{11}} \right) \times 100$$

- $V_S$  = Titrant volume consumed by the *Sample* (mL)  
 $V_B$  = Titrant volume consumed by the *Blank* (mL)  
 $M$  = Titrant molarity (mmol/mL)  
 $F$  = equivalency factor, 430.4 mg/mmol  
 $W$  = *Sample* weight (mg)  
 $M_{12}$  = molecular weight of calcium gluconate monohydrate, 448.4  
 $M_{11}$  = molecular weight of calcium gluconate anhydrous, 430.4

#### Acceptance criteria

**Anhydrous:** 98.0%–102.0% on the dried basis

**Monohydrate:** 99.0%–101.0% where labeled as intended for use in preparing injectable dosage forms; 98.5%–102.0% where labeled as not intended for use in preparing injectable dosage forms

#### IMPURITIES

##### • ARSENIC (211), Method I

**Test preparation:** Dissolve 1.0 g in a mixture of 10 mL of hydrochloric acid and 20 mL of water, and dilute with water to 55 mL.

**Analysis:** Proceed as directed in the chapter, except to omit the addition of 20 mL of 7 N sulfuric acid.

**Acceptance criteria:** NMT 3 ppm

- **CHLORIDE AND SULFATE (221), Chloride:** A 1.0-g portion shows no more chloride than corresponds to 0.07 mL of 0.020 N hydrochloric acid (0.005%). Where it is labeled as not intended for use in the preparation of injectable dosage forms, a 1.0-g portion shows no more chloride than corresponds to 1 mL of 0.020 N hydrochloric acid (0.07%).
- **CHLORIDE AND SULFATE (221), Sulfate:** A 2.0-g portion dissolved in boiling water shows no more sulfate than corresponds to 0.1 mL of 0.020 N sulfuric acid (0.005%). Where it is labeled as not intended for use in the preparation of injectable dosage forms, a 2.0-g portion dissolved in boiling water shows no more sulfate than corresponds to 1 mL of 0.020 N sulfuric acid (0.05%).

#### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 10 ppm; NMT 20 ppm where Calcium Gluconate is labeled as not intended for use in the preparation of injectable dosage forms. (Official 1-Jan-2018)

##### • LIMIT OF IRON

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

**Internal standard solution:** 2 µg/mL of yttrium in 10% nitric acid, prepared from commercially prepared yttrium standard suitable for ICP-OES. [NOTE—The yttrium concentration can be varied to optimize the analysis.]

**Standard solutions:** 0.05, 0.1, 0.2, and 0.4 µg/mL of iron in 10% nitric acid, prepared from commercially prepared iron standard suitable for ICP-OES

**Sample solution:** 40 mg/mL of Calcium Gluconate in 10% nitric acid

**Blank solution:** 10% Nitric acid solution

#### Instrumental conditions

(See *Plasma Spectrochemistry* (730).)

**Mode:** Inductively coupled plasma-optical emission spectroscopy (ICP-OES)

**Emission wavelengths:** 239.562 nm or optimized wavelength for iron; 371.029 nm or optimized wavelength for yttrium

#### System suitability

**Samples:** *Internal standard solution*, *Standard solutions*, and *Blank solution*

Instrument performance must be verified to conform to the manufacturer's specifications for resolution and sensitivity. Before analyzing samples, the instrument must pass a suitable performance check. Generate the calibration curve using the *Blank solution* and *Standard solutions* as follows. Scan the *Internal standard solution* while running the *Blank solution* to measure the intensity of the yttrium emission. Hold this value constant throughout the remainder of the test. Separately scan the *Blank solution*; *Standard solutions* of 0.05, 0.1, 0.2, and 0.4 µg/mL of iron; and *Internal standard solution*. [NOTE—Add the *Internal standard solution* via an in-line mixing chamber.] Normalize the yttrium intensity to the value of the *Internal standard solution*. Apply this normalization factor to the iron intensity, which is then referred to as the corrected iron intensity. Construct a calibration curve by plotting the corrected iron intensity versus the known concentrations, in µg/mL, of the iron: the linear regression coefficient is NLT 0.999.

#### Analysis

**Sample:** *Sample solution*

Similarly, analyze the *Sample solution* on the ICP. Plot the intensity of the emission of the *Sample solution* on the calibration curve. Obtain the concentration of iron,  $C$ , in µg/mL, in the *Sample solution* through the calibration curve.

Calculate the content, in µg/g (ppm), of iron in the portion of Calcium Gluconate taken:

$$\text{Result} = (C \times V)/W$$

- $C$  = concentration of iron in the *Sample solution* obtained from the calibration curve (µg/mL)  
 $V$  = volume of the *Sample solution* (mL)  
 $W$  = weight of Calcium Gluconate taken to prepare the *Sample solution* (g)

**Acceptance criteria:** NMT 5 ppm

##### • LIMIT OF MAGNESIUM AND ALKALI METALS

[NOTE—Calcium Gluconate labeled as not intended for use in preparing injectable dosage forms is exempt from this requirement.]

**Sample:** 1.0 g

**Analysis:** Dissolve the *Sample* completely in 100 mL of boiling water. Add 10 mL of ammonium chloride TS, 1 mL of ammonium hydroxide, and 50 mL of hot (maintained at 70°–80°) ammonium oxalate TS. Allow to stand for 4 h, dilute with water to 200 mL, and filter. Evaporate 100 mL of the filtrate to dryness, and ignite to constant weight.

**Acceptance criteria:** NMT 0.4%; the weight of the residue does not exceed 2 mg.

##### • LIMIT OF PHOSPHATE

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

**Standard stock solution 1:** 0.716 mg/mL of monobasic potassium phosphate

**Standard stock solution 2:** Dilute 1.0 mL of *Standard stock solution 1* with water to 100 mL.

**Standard solution:** Dilute 2.0 mL of *Standard stock solution 2* with water to 100 mL.

**Sample stock solution:** To 10.0 g of Calcium Gluconate add 90 mL of hot water (70°–80°), and heat to boiling, with swirling, for 10 s to obtain a clear solution.

**Sample solution:** Dilute 1 mL of the hot *Sample stock solution* with water to 100 mL.



**Analysis**

**Samples:** *Standard solution* and *Sample solution*

To the *Standard solution* and *Sample solution* add 4 mL of sulfomolybdic acid TS, and mix. To both solutions add 0.1 mL of a freshly prepared mixture of 3 N hydrochloric acid and stronger acid stannous chloride TS (10:1), and mix.

**Acceptance criteria:** NMT 0.01%; after 10 min any color in the *Sample solution* is not more intense than that in the *Standard solution*.

• **LIMIT OF OXALATE**

[NOTE—Calcium Gluconate labeled as not intended for use in the preparation of injectable dosage forms is exempt from this requirement.]

[NOTE—Use deionized water where water is indicated.]

**Solution A:** 0.0125 M sulfuric acid in water

**Solution B:** Dilute 1 mL of hydrochloric acid with water to 1200 mL.

**Mobile phase:** 0.0017 M sodium bicarbonate and 0.0018 M sodium carbonate in water

**Standard solution:** 1.5 µg/mL of sodium oxalate in *Solution B*

**Sample solution:** 20 mg/mL of Calcium Gluconate in *Solution B*. Sonicate if necessary.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** Ion chromatography

**Detector:** Conductance

**Columns**

**Guard:** 4-mm × 5-cm; 15-µm packing L12

**Analytical:** 4-mm × 25-cm; 15-µm packing L12

**Anion suppressor:** The micromembrane anion suppressor column is connected in series with the guard and analytical columns. The anion suppressor column is equipped with a micromembrane that separates *Mobile phase* from *Solution A* flowing countercurrent to *Mobile phase* at a rate of about 7 mL/min. [NOTE—Condition the system for about 15 min with *Mobile phase* at a flow rate of 2 mL/min.]

**Flow rate:** 2 mL/min

**Injection volume:** 50 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 2500 theoretical plates

**Tailing factor:** NMT 1.2

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of oxalate in the portion of Calcium Gluconate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times F \times 100$$

$r_U$  = peak response of oxalate from the *Sample solution*

$r_S$  = peak response of oxalate from the *Standard solution*

$C_S$  = concentration of sodium oxalate in the *Standard solution* (µg/mL)

$C_U$  = concentration of Calcium Gluconate in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of oxalate, 88.03

$M_{r2}$  = molecular weight of sodium oxalate, 134.00

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** NMT 0.01%

• **REDUCING SUBSTANCES**

**Sample:** 1.0 g of Calcium Gluconate

**Blank:** 20 mL of water

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Residual titration

**Titrant:** 0.1 N iodine VS

**Back titrant:** 0.1 N sodium thiosulfate VS

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a 250-mL conical flask, dissolve in 20 mL of hot water, cool, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid. Titrate with the *Back titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the *Blank* determination.

Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = [(V_B - V_S) \times N \times F/W] \times 100$$

$V_B$  = *Back titrant* volume consumed by the *Blank* (mL)

$V_S$  = *Back titrant* volume consumed by the *Sample* (mL)

$N$  = *Back titrant* normality (mEq/mL)

$F$  = equivalency factor, 27 mg/mEq

$W$  = *Sample* weight (mg)

**Acceptance criteria:** NMT 1.0%

**SPECIFIC TESTS**

• **LOSS ON DRYING** (731)

**Analysis:** Dry at 105° for 16 h.

**Acceptance criteria**

**Anhydrous:** NMT 3.0%

**Monohydrate:** NMT 1.0%, where labeled as intended for use in preparing injectable dosage forms; NMT 2.0%, where labeled as not intended for use in preparing injectable dosage forms

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** Label it to indicate whether it is anhydrous or monohydrate. Where the quantity of calcium gluconate is indicated in the labeling of any solution containing Calcium Gluconate, this shall be understood to be in terms of anhydrous calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ). Calcium Gluconate intended for use in preparing injectable dosage forms is so labeled. Calcium Gluconate not intended for use in preparing injectable dosage forms is so labeled; in addition, it may be labeled also as intended for use in preparing oral dosage forms.

• **USP REFERENCE STANDARDS** (11)

USP Calcium Gluconate Anhydrous RS

USP Calcium Gluconate Monohydrate RS

## Calcium Gluconate Injection

**DEFINITION**

Calcium Gluconate Injection is a sterile solution of Calcium Gluconate in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of total calcium. The calcium is in the form of calcium gluconate, except that a small amount may be replaced with an equal amount of calcium in the form of Calcium Saccharate, or other suitable calcium salts, for the purpose of stabilization. It may contain sodium hydroxide added for adjustment of the pH.

Injection intended for veterinary use only may be prepared from Calcium Gluconate solubilized with Boric Acid, or from Gluconolactone, Boric Acid, and Calcium Carbonate.



**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHY**

**Standard solution:** 10 mg/mL of USP Potassium Gluconate RS

**Sample solution:** Dilute a volume of injection, if necessary, with water to obtain a concentration of 10 mg/mL of calcium gluconate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 5 µL

**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 N sulfuric acid to volume, and mix.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the *Spray reagent*. Heat the plate at 110° for about 10 min.

**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and  $R_f$  value to that of the *Standard solution*.

• **B. IDENTIFICATION TESTS—GENERAL, Calcium (191)**

**Sample solution:** Dilute a volume of Injection with water to obtain a concentration of 20 mg/mL of calcium gluconate.

**Acceptance criteria:** Meets the requirements

**ASSAY**• **PROCEDURE**

**Sample:** Accurately measured volume of Injection, equivalent to 46.5 mg of calcium

**Blank:** 150 mL of water containing 2 mL of 3 N hydrochloric acid

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Add 2 mL of 3 N hydrochloric acid to the *Sample*, and dilute with water to 150 mL. While stirring, add 20 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform a blank determination.

Calculate the percentage of the labeled amount of total calcium in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F/W] \times 100\}$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$M$  = *Titrant* molarity (mmol/mL)

$F$  = equivalency factor, 40.08 mg/mmol

$W$  = weight of calcium in the *Sample* (mg)

**Acceptance criteria:** 95.0%–105.0%

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.17 USP Endotoxin Units/mg of calcium gluconate
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **PH (791):** 6.0–8.2; 2.5–4.5 where labeled as intended for veterinary use only and as containing boric acid
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS (1):** Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass.

- **LABELING:** Label the Injection to indicate its content, if any, of added calcium salts, calculated as percentage of calcium in the Injection. The label states the total osmolar concentration in mOsmol/L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol/mL. The labeling indicates that the Injection must be clear at the time of use, and that if crystallization has occurred, warming may redissolve the precipitate. Injection intended for veterinary use only is so labeled. If Injection contains boric acid, it is so labeled.

• **USP REFERENCE STANDARDS (11)**

USP Endotoxin RS

USP Potassium Gluconate RS

**Calcium Gluconate Tablets****DEFINITION**

Calcium Gluconate Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of calcium gluconate ( $\text{C}_{12}\text{H}_{22}\text{CaO}_{14}$ ).

**IDENTIFICATION**• **A. IDENTIFICATION TESTS—GENERAL, Calcium (191)**

**Sample stock solution:** A warm, filtered solution in water, equivalent to 100 mg/mL of calcium gluconate from powdered Tablets

**Sample solution:** Equivalent to 20 mg/mL of calcium gluconate from a dilution of the *Sample stock solution*

**Acceptance criteria:** Meet the requirements

• **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**

**Standard solution:** 10 mg/mL of USP Potassium Gluconate RS

**Sample solution:** Equivalent to 10 mg/mL of calcium gluconate from a dilution of the *Sample stock solution* obtained from *Identification test A*

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 5 µL

**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)

**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Develop until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for about 10 min.

**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and  $R_f$  value to that of the *Standard solution*.

**ASSAY**• **PROCEDURE**

**Sample:** A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 500 mg of calcium gluconate

**Blank:** Proceed as directed in the *Analysis*, without the *Sample*.



**Titrimetric system**(See *Titrimetry* (541).)**Mode:** Direct titration**Titrant:** 0.05 M edetate disodium VS**Indicator:** Hydroxy naphthol blue, 300 mg**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a suitable crucible. Ignite, gently at first, until free from carbon. Cool the crucible. Add 10 mL of water, and dissolve the residue by adding sufficient 3 N hydrochloric acid, dropwise, to achieve complete solution. Transfer the solution to a suitable container, and add about 150 mL of water. While stirring, preferably with a magnetic stirrer, add 20 mL of *Titrant* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide, then add the *Indicator*. Continue the titration to a blue endpoint. Perform a *Blank* determination.

Calculate the percentage of the labeled amount of calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ) in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$M$  = actual molarity of the *Titrant* (mM/mL)

$F$  = equivalency factor, 430.4 (mg/mM)

$W$  = nominal weight of calcium gluconate in the *Sample* (mg)

**Acceptance criteria:** 95.0%–105.0%

**PERFORMANCE TESTS**• **DISSOLUTION** (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Standard solution:** Solution having a known concentration of calcium in *Medium*

**Sample solution:** Filtered portion of the solution under test, suitably diluted with *Medium* if necessary

**Instrumental conditions**

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Analytical wavelength:** 422.8 nm

**Lamp:** Calcium hollow-cathode

**Flame:** Air–acetylene

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S \times D \times V/L) \times (M_r/A_r) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of calcium in the *Standard solution* (mg/mL)

$D$  = dilution factor for the *Sample solution*

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

$M_r$  = molecular weight of calcium gluconate, 430.4

$A_r$  = atomic weight of calcium, 40.078

**Tolerances:** NLT 75% (Q) of the labeled amount of calcium gluconate ( $C_{12}H_{22}CaO_{14}$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS** (11)

USP Potassium Gluconate RS

**Calcium Hydroxide**

$Ca(OH)_2$

74.09

Calcium hydroxide [1305-62-0].

**DEFINITION**

Calcium Hydroxide contains NLT 95.0% and NMT 100.5% of calcium hydroxide [ $Ca(OH)_2$ ].

**IDENTIFICATION**• **A.**

**Sample solution:** Mix with three to four times its weight of water.

**Acceptance criteria:** It forms a smooth magma. The clear supernatant from the magma is alkaline to litmus.

• **B. IDENTIFICATION TESTS—GENERAL, Calcium** (191)

**Sample solution:** Mix 1 g with 20 mL of water, and add sufficient 6 N acetic acid to effect the solution.

**Acceptance criteria:** Meets the requirements

**ASSAY**• **PROCEDURE**

**Sample solution:** To 1.5 g of Calcium Hydroxide in a beaker, gradually add 30 mL of 3 N hydrochloric acid. When dissolved, transfer the solution to a 500-mL volumetric flask, and rinse the beaker thoroughly, adding the rinsings to the flask. Dilute with water to volume, and mix. Transfer 50 mL of the solution into a suitable container, and add 100 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue.

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Titrate the *Sample solution* with *Titrant* to a blue endpoint. Each mL of *Titrant* is equivalent to 3.705 mg of calcium hydroxide [ $Ca(OH)_2$ ].

**Acceptance criteria:** 95.0%–100.5%

**IMPURITIES****Delete the following:**• **HEAVY METALS** (231)

**Test preparation:** Dissolve 2.0 g in 20 mL of 3 N hydrochloric acid, and evaporate on a steam bath to dryness. Dissolve the residue in 20 mL of water, and filter. Dilute the filtrate with water to 40 mL. To 20 mL of this solution add 1 mL of 0.1 N hydrochloric acid, then add water to make 25 mL.

**Acceptance criteria:** NMT 20 ppm • (Official 1-Jan-2018)

• **LIMIT OF MAGNESIUM AND ALKALI SALTS**

**Sample solution:** Dissolve 0.50 g in a mixture of 30 mL of water and 10 mL of 3 N hydrochloric acid.

**Analysis:** Heat the solution, and boil for 1 min. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Add immediately to the warm mixture 2 drops of methyl red TS and then 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline. Cool to room temperature, transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Filter, and to 50 mL of the clear filtrate in a platinum dish add 0.5 mL of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammo-



nium salts. Finally, ignite the residue to constant weight.

Acceptance criteria: NMT 12 mg (4.8%)

• **LIMIT OF ACID-INSOLUBLE SUBSTANCES**

**Sample solution:** Dissolve 2.0 g in 30 mL of 4 N hydrochloric acid, and heat to boiling.

**Analysis:** Filter the mixture, wash the residue with hot water, and ignite.

Acceptance criteria: 0.5%; the weight of the residue is NMT 10 mg.

• **CARBONATE**

**Sample solution:** Mix 2 g with 50 mL of water.

**Analysis:** Add an excess of 3 N hydrochloric acid to the *Sample solution*.

Acceptance criteria: The *Analysis* does not cause more than a slight effervescence.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

## Calcium Hydroxide Topical Solution

» Calcium Hydroxide Topical Solution is a solution containing, in each 100 mL, not less than 140 mg of calcium hydroxide  $[\text{Ca}(\text{OH})_2]$ .

Prepare Calcium Hydroxide Topical Solution as follows (see *Pharmaceutical Compounding—Non-sterile Preparations* (795)):

Calcium Hydroxide .....	3 g
Purified Water .....	1000 mL

Add the Calcium Hydroxide to 1000 mL of cool Purified Water, and agitate the mixture vigorously and repeatedly during 1 hour. Allow the excess calcium hydroxide to settle. Dispense only the clear supernatant.

**NOTE**—The solubility of calcium hydroxide, which varies with the temperature at which the solution is stored, is about 170 mg per 100 mL at 15° and less at a higher temperature. The official concentration is based upon a temperature of 25°.

The undissolved portion of the mixture is not suitable for preparing additional quantities of Calcium Hydroxide Topical Solution.

**Packaging and storage**—Preserve in well-filled, tight containers, at a temperature not exceeding 25°.

**Identification**—

A: It absorbs carbon dioxide from the air, a film of calcium carbonate forming on the surface of the liquid.

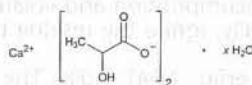
B: When heated, it becomes turbid, owing to the separation of calcium hydroxide.

C: It responds to the tests for *Calcium* (191).

**Alkalies and their carbonates**—A portion of it, saturated with carbon dioxide and subsequently boiled, is neutral in reaction.

**Assay**—Pipet 100 mL of Topical Solution into a suitable container, add 50 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue, and titrate with 0.05 M edetate disodium VS to a blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 3.705 mg of calcium hydroxide  $[\text{Ca}(\text{OH})_2]$ .

## Calcium Lactate



$\text{C}_6\text{H}_{10}\text{CaO}_6 \cdot 5\text{H}_2\text{O}$  308.30

$\text{C}_6\text{H}_{10}\text{CaO}_6$  218.22

Propanoic acid, 2-hydroxy-, calcium salt (2:1), hydrate;

Calcium lactate (1:2) hydrate [41372-22-9].

Calcium lactate (1:2) pentahydrate [5743-47-5].

Anhydrous [814-80-2].

**DEFINITION**

Calcium Lactate contains NLT 98.0% and NMT 101.0% of calcium lactate ( $\text{C}_6\text{H}_{10}\text{CaO}_6$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** Meets the requirements
- **B. INFRARED ABSORPTION (197K)**

**ASSAY**

• **PROCEDURE**

**Sample:** Weighed portion of Calcium Lactate equivalent to 350 mg of  $\text{C}_6\text{H}_{10}\text{CaO}_6$

**Blank:** 150 mL of water and 2 mL of 3 N hydrochloric acid

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in a mixture of water and 3 N hydrochloric acid (150:2). While stirring with a magnetic stirrer, add 30 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.

Calculate the percentage of calcium lactate ( $\text{C}_6\text{H}_{10}\text{CaO}_6$ ) in the *Sample* taken:

$$\text{Result} = \left\{ \frac{(V_S - V_B) \times M \times F}{W} \right\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$M$  = *Titrant* molarity (mM/mL)

$F$  = equivalency factor, 218.2 mg/mM

$W$  = *Sample* weight (mg)

Acceptance criteria: 98.0%–101.0% on the dried basis

**IMPURITIES**

**Delete the following:**

• **HEAVY METALS (231)**

**Test preparation:** Dissolve 1 g in 2.5 mL of 1 N acetic acid, and dilute with water to 25 mL.

Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

• **LIMIT OF MAGNESIUM AND ALKALI SALTS**

**Sample:** 1.0 g Calcium Lactate

**Analysis:** Mix the *Sample* with 40 mL of water, carefully add 1 mL of hydrochloric acid, and heat the solution to boiling. Rapidly add 40 mL of oxalic acid TS, and stir vigorously until precipitation is well established. Add immediately to the warm mixture 2 drops of methyl red TS and then 6 N ammonium hydroxide, dropwise, until the mixture is just alkaline. Cool to room temperature, transfer to a 100-mL graduated cylinder, dilute with water to 100 mL, mix, and allow to stand for 4 h or overnight. Filter, and to 50 mL of the clear filtrate in a



platinum dish add 0.5 mL of sulfuric acid. Evaporate the mixture on a steam bath to a small volume. Carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of ammonium salts. Finally, ignite the residue to constant weight.

**Acceptance criteria:** NMT 1.0%: The weight of the residue does not exceed 5.0 mg.

• **VOLATILE FATTY ACID**

**Sample solution:** Stir 500 mg of Calcium Lactate with 1 mL of sulfuric acid, and warm.

**Acceptance criteria:** The mixture does not emit an odor of volatile fatty acid.

**SPECIFIC TESTS**

• **ACIDITY**

**Sample solution:** 50 mg/mL

**Analysis:** Titrate 20 mL of *Sample solution* with 0.10 N sodium hydroxide, using phenolphthalein TS as the indicator.

**Acceptance criteria:** NMT 0.50 mL is required for neutralization, equivalent to NMT 0.45% as lactic acid.

• **LOSS ON DRYING (731)**

**Sample:** 1–2 g

**Analysis:** Distribute the *Sample* evenly in a suitable weighing dish to a depth of NMT 3 mm, and dry at 120° for 4 h.

**Acceptance criteria:** See Table 1.

Table 1

Form	Loss on Drying (%)
Pentahydrate	22.0–27.0
Trihydrate	15.0–20.0
Monohydrate	5.0–8.0
Anhydrous	NMT 3.0

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label indicates whether it is the dried form or is hydrous; if the latter, the label indicates the degree of hydration. Where the quantity of Calcium Lactate is indicated in the labeling of any solution containing Calcium Lactate, this shall be understood to be in terms of calcium lactate pentahydrate ( $\text{C}_6\text{H}_{10}\text{CaO}_6 \cdot 5\text{H}_2\text{O}$ ).
- **USP REFERENCE STANDARDS (11)**  
USP Calcium Lactate RS

## Calcium Lactate Tablets

**DEFINITION**

Calcium Lactate Tablets contain NLT 94.0% and NMT 106.0% of the labeled amount of calcium lactate pentahydrate ( $\text{C}_6\text{H}_{10}\text{CaO}_6 \cdot 5\text{H}_2\text{O}$ ).

[NOTE—An equivalent amount of Calcium Lactate with less water of hydration may be used in place of calcium lactate pentahydrate ( $\text{C}_6\text{H}_{10}\text{CaO}_6 \cdot 5\text{H}_2\text{O}$ ) in preparing Calcium Lactate Tablets.]

**IDENTIFICATION**

• **A. IDENTIFICATION TESTS—GENERAL, Calcium (191)**

**Sample solution:** A filtered solution, equivalent to 50 mg/mL of calcium lactate pentahydrate from powdered Tablets

**Acceptance criteria:** Meet the requirements

• **B. IDENTIFICATION TESTS—GENERAL, Lactate (191)**

**Sample solution:** A filtered solution, equivalent to 50 mg/mL of calcium lactate pentahydrate from powdered Tablets

**Acceptance criteria:** Meet the requirements

**ASSAY**

• **PROCEDURE**

**Sample:** A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 350 mg of calcium lactate pentahydrate ( $\text{C}_6\text{H}_{10}\text{CaO}_6 \cdot 5\text{H}_2\text{O}$ )

**Blank:** Proceed as directed in the *Analysis* without the *Sample*.

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Indicator:** Hydroxy naphthol blue, 300 mg

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a suitable container, and add 150 mL of water and 2 mL of 3 N hydrochloric acid. Stir, using a magnetic stirrer, for 3–5 min. While stirring, add 15 mL of *Titrant* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform a *Blank* determination.

Calculate the percentage of the labeled amount of calcium lactate pentahydrate ( $\text{C}_6\text{H}_{10}\text{CaO}_6 \cdot 5\text{H}_2\text{O}$ ) in the portion of Tablets taken:

$$\text{Result} = \left[ \frac{(V_s - V_b) \times M \times F}{W} \right] \times 100$$

$V_s$  = *Titrant* volume consumed by the *Sample* (mL)

$V_b$  = *Titrant* volume consumed by the *Blank* (mL)

$M$  = actual molarity of the *Titrant* (mM/mL)

$F$  = equivalency factor, 308.4 (mg/mM)

$W$  = nominal weight of calcium lactate pentahydrate in the *Sample* taken (mg)

**Acceptance criteria:** 94.0%–106.0%

**PERFORMANCE TESTS**

• **DISSOLUTION, Procedure for a Pooled Sample (711)**

**Medium:** Water; 500 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Analysis:** Determine the amount of calcium lactate pentahydrate ( $\text{C}_6\text{H}_{10}\text{CaO}_6 \cdot 5\text{H}_2\text{O}$ ) dissolved, as directed in the *Assay*, making any necessary modifications.

**Tolerances:** NLT 75% (Q) of the labeled amount of calcium lactate pentahydrate ( $\text{C}_6\text{H}_{10}\text{CaO}_6 \cdot 5\text{H}_2\text{O}$ ) is dissolved.

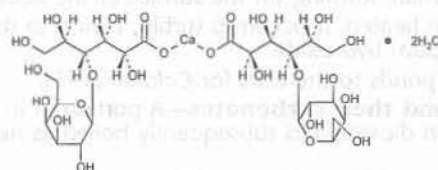
• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **LABELING:** The quantity of Calcium Lactate stated in the labeling is in terms of calcium lactate pentahydrate.

## Calcium Lactobionate



$\text{C}_{24}\text{H}_{42}\text{CaO}_{24} \cdot 2\text{H}_2\text{O}$  790.68

D-Gluconic acid, 4-O-β-D-galactopyranosyl-, calcium salt (2:1), dihydrate;

Lactobionic acid, calcium salt (2:1), dihydrate;

Calcium lactobionate (1:2), dihydrate [110638-68-1].



**DEFINITION**

Calcium Lactobionate contains NLT 96.0% and NMT 102.0% of calcium lactobionate ( $C_{24}H_{42}CaO_{24} \cdot 2H_2O$ ).

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL**, Calcium (191): A 20 mg/mL solution meets the requirements.
- **B. INFRARED ABSORPTION** (197K)
- **C. THIN LAYER CHROMATOGRAPHY**  
 Standard solution: 10 mg/mL of USP Calcium Lactobionate RS in water  
 Sample solution: 10 mg/mL of Calcium Lactobionate in water  
**Chromatographic system**  
 (See Chromatography (621), Thin-Layer Chromatography.)  
 Mode: TLC  
 Adsorbent: 0.25-mm layer of chromatographic silica gel  
 Application volume: 5  $\mu$ L  
 Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)  
 Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, dilute with 2 N sulfuric acid to volume, and mix.  
**Analysis:**  
 Samples: Standard solution and Sample solution  
 Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 100° for 20 min. Allow to cool, and spray with the Spray reagent. Heat the plate at 110° for about 10 min.  
**Acceptance criteria:** The principal spot of the Sample solution corresponds in color, size, and  $R_f$  value to that of the Standard solution.

**ASSAY**• **PROCEDURE**

**Sample:** 800 mg of Calcium Lactobionate  
**Blank:** 150 mL of water and 2 mL of 3 N hydrochloric acid  
**Titrimetric system**  
 (See Titrimetry (541).)  
**Mode:** Direct titration  
**Titrant:** 0.05 M edetate disodium VS  
**Endpoint detection:** Visual  
**Analysis:** Dissolve the Sample in a mixture of water and 3 N hydrochloric acid (150:2). While stirring with a magnetic stirrer, add 15 mL of Titrant from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the Blank determination.  
 Calculate the percentage of calcium lactobionate ( $C_{24}H_{42}CaO_{24} \cdot 2H_2O$ ) in the Sample taken:

$$\text{Result} = \{[(V_s - V_b) \times M \times F]/W\} \times 100$$

$V_s$  = Titrant volume consumed by the Sample (mL)  
 $V_b$  = Titrant volume consumed by the Blank (mL)  
 $M$  = Titrant molarity (mM/mL)  
 $F$  = equivalency factor, 790.7 mg/mmol  
 $W$  = Sample weight (mg)  
**Acceptance criteria:** 96.0%–102.0%

**IMPURITIES**• **HALIDES**

**Standard solution:** 0.7 mL of 0.020 N hydrochloric acid  
**Sample:** 1.2 g  
**Analysis:** Proceed as directed for Chloride and Sulfate (221), Chloride.

**Acceptance criteria:** NMT 0.04%

- **CHLORIDE AND SULFATE**, Sulfate (221)  
**Standard solution:** 1 mL of 0.020 N sulfuric acid  
**Sample:** 2.0 g  
**Analysis:** Dissolve the Sample in boiling water.  
**Acceptance criteria:** NMT 0.05%

**Delete the following:**• **HEAVY METALS** (231)

**Test preparation:** Mix 1 g in 4 mL of 1.2 N hydrochloric acid. Add water to make 25 mL, warm gently until dissolved, and cool to room temperature.

**Acceptance criteria:** NMT 20 ppm (Official 1-Jan-2018)

• **REDUCING SUBSTANCES**

**Sample:** 1.0 g of Calcium Lactobionate

**Blank:** 20 mL of water

**Titrimetric system**

(See Titrimetry (541).)

**Mode:** Residual titration

**Titrant:** 0.1 N iodine VS

**Back-titrant:** 0.1 N sodium thiosulfate VS

**Endpoint detection:** Visual

**Analysis:** Transfer the Sample to a 250-mL conical flask, dissolve in the Blank, and add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of Titrant, and 10 mL of 3 N hydrochloric acid, and titrate with Back-titrant, adding 3 mL of starch TS as the endpoint is approached. Perform the Blank determination. Calculate the percentage of reducing substances (as dextrose) in the Sample taken:

$$\text{Result} = \{[(V_b - V_s) \times N \times F]/W\} \times 100$$

$V_b$  = Back-titrant volume consumed by the Blank (mL)

$V_s$  = Back-titrant volume consumed by the Sample (mL)

$N$  = Back-titrant normality (mEq/mL)

$F$  = equivalency factor, 27 mg/mEq

$W$  = Sample weight (mg)

**Acceptance criteria:** NMT 1.0%

**SPECIFIC TESTS**• **OPTICAL ROTATION**, Specific Rotation (781S)

**Sample solution:** 100 mg/mL

**Acceptance criteria:** +22.0° to +26.5°

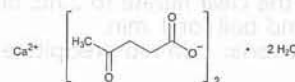
• **pH** (791)

**Sample solution:** 50 mg/mL

**Acceptance criteria:** 5.4–7.4

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Calcium Lactobionate RS

**Calcium Levulinate**

$C_{10}H_{14}CaO_6 \cdot 2H_2O$

306.32

$C_{10}H_{14}CaO_6$

270.30

Pentanoic acid, 4-oxo-, calcium salt (2:1), dihydrate;  
 Calcium levulinate (1:2) dihydrate [5743-49-7].  
 Anhydrous [591-64-0].



**DEFINITION**

Calcium Levulinate contains NLT 97.5% and NMT 100.5% of calcium levulinate ( $C_{10}H_{14}CaO_6$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191):** A 100 mg/mL solution meets the requirements.

- **B.**

**Sample solution:** 0.5 g in 5 mL of water

**Analysis:** To the *Sample solution* add 5 mL of 1 N sodium hydroxide, and filter. To the filtrate add 5 mL of iodine TS.

**Acceptance criteria:** A precipitate of iodoform is produced.

**ASSAY**

- **PROCEDURE**

**Sample:** 600 mg of Calcium Levulinate

**Blank:** 150 mL of water containing 2 mL of 3 N hydrochloric acid

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. While stirring with a magnetic stirrer, add 30 mL of *Titrant* from the titration buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform the *Blank* determination.

Calculate the percentage of calcium levulinate ( $C_{10}H_{14}CaO_6$ ) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F/W] \times 100\}$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$M$  = *Titrant* molarity (mM/mL)

$F$  = equivalency factor, 270.3 mg/mM

$W$  = *Sample* weight (mg)

**Acceptance criteria:** 97.5%–100.5% on the dried basis

**IMPURITIES**

- **CHLORIDE AND SULFATE, Chloride (221)**

**Standard:** 1.0 mL of 0.020 N hydrochloric acid

**Sample:** 1.0 g

**Acceptance criteria:** NMT 0.07%

- **CHLORIDE AND SULFATE, Sulfate (221)**

**Standard:** 1.0 mL of 0.020 N sulfuric acid

**Sample:** 2.0 g

**Acceptance criteria:** NMT 0.05%

**Delete the following:**

- **HEAVY METALS (231):** NMT 20 ppm (Official 1-Jan-2018)

- **REDUCING SUGARS**

**Sample:** 0.50 g

**Analysis:** Dissolve the *Sample* in 10 mL of water, add 2 mL of 3 N hydrochloric acid, boil for about 2 min, and cool. Add 5 mL of sodium carbonate TS, allow to stand for 5 min, dilute with water to 20 mL, and filter. Add 5 mL of the clear filtrate to 2 mL of alkaline cupric tartrate TS, and boil for 1 min.

**Acceptance criteria:** No red precipitate is formed immediately.

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE, Class I (741):**

119°–125°

- **pH (791)**

**Sample solution:** 100 mg/mL

**Acceptance criteria:** 7.0–8.5

- **LOSS ON DRYING (731):** Dry a sample at a pressure not exceeding 5 mm of mercury at 60° for 5 h; it loses 10.5%–12.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

**Calcium Levulinate Injection**

» Calcium Levulinate Injection is a sterile solution of Calcium Levulinate in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{10}H_{14}CaO_6 \cdot 2H_2O$ .

**Packaging and storage—**Preserve in single-dose containers, preferably of Type I glass.

**Labeling—**The label states the total osmolar concentration in mOsmol per L. Where the contents are less than 100 mL, or where the label states that the Injection is not for direct injection but is to be diluted before use, the label alternatively may state the total osmolar concentration in mOsmol per mL.

**USP Reference standards (11)—**

USP Endotoxin RS

**Identification—**It responds to the *Identification* tests under *Calcium Levulinate*.

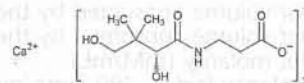
**Bacterial Endotoxins Test (85)—**It contains not more than 35.70 USP Endotoxin Units per mg of calcium levulinate.

**pH (791):** between 6.0 and 8.0.

**Particulate Matter in Injections (788):** meets the requirements for small-volume injections.

**Other requirements—**It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay—**Transfer an accurately measured volume of Injection, equivalent to about 600 mg of calcium levulinate, to a 400-mL beaker, add 2 mL of hydrochloric acid, and proceed as directed in the *Assay* under *Calcium Levulinate*, beginning with "While stirring with a magnetic stirrer." Each mL of 0.05 M edetate disodium is equivalent to 15.32 mg of  $C_{10}H_{14}CaO_6 \cdot 2H_2O$ .

**Calcium Pantothenate**

$C_{18}H_{32}CaN_2O_{10}$  476.53

$\beta$ -Alanine, N-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-, calcium salt (2:1), (R)-;

Calcium D-pantothenate (1:2) [137-08-6].

**DEFINITION**

Calcium Pantothenate is the calcium salt of the dextrorotatory isomer of pantothenic acid. It contains NLT 98.0% and NMT 102.0% of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ), calculated on the dried basis.



**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL**, Calcium (191): A 50-mg/mL solution meets the requirements.
- **C. OPTICAL ROTATION**, Specific Rotation (781S)  
Sample solution: 50 mg/mL in water  
Acceptance criteria: +25.0° to +27.5°

**ASSAY**• **PROCEDURE**

**Buffer solution:** Dissolve 3.2 g of monobasic sodium phosphate in 1 L of water; adjust with 1 N sodium hydroxide to a pH of 5.5.

**Mobile phase:** Acetonitrile and Buffer solution (2:98)

**System suitability solution:** 0.1 mg/mL of USP Calcium Pantothenate RS and 0.5 mg/mL each of USP β-Alanine RS, USP Sodium D-Pantoate RS, and USP Pantolactone RS in water

**Standard solution:** 0.5 mg/mL of USP Calcium Pantothenate RS in water

**Sample solution:** 0.5 mg/mL of Calcium Pantothenate in water

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Column temperature:** 35°

**Flow rate:** 2 mL/min

**Injection volume:** 10 μL

**System suitability**

**Samples:** System suitability solution and Standard solution

[NOTE—The relative retention times for β-alanine, pantoic acid, pantothenic acid, and pantolactone are 0.3, 0.6, 1.0, and 1.9 min, respectively.]

**Suitability requirements**

**Resolution:** NLT 5.0 between pantothenic acid and pantoic acid peaks, System suitability solution

**Tailing factor:** NMT 2.0, Standard solution

**Relative standard deviation:** NMT 2.0%, Standard solution

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ) in the portion of Calcium Pantothenate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Calcium Pantothenate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Calcium Pantothenate in the Sample solution (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

**OTHER COMPONENTS**• **CONTENT OF CALCIUM**

**Sample:** 800 mg of Calcium Pantothenate

**Blank:** 150 mL of water containing 2 mL of 3 N hydrochloric acid

**Titrimetric system**

(See Titrimetry (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the Sample in 150 mL of water containing 2 mL of 3 N hydrochloric acid. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and titrate with Titrant to a distinct blue endpoint. Perform the Blank determination.

Calculate the percentage of calcium (Ca) in the Sample taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F] / W\} \times 100$$

$V_S$  = Titrant volume consumed by the Sample (mL)

$V_B$  = Titrant volume consumed by the Blank (mL)

$M$  = actual molarity of the Titrant (mmol/mL)

$F$  = equivalency factor, 40.08 mg/mmol

$W$  = Sample weight (mg)

Acceptance criteria: 8.2%–8.6% on the dried basis

**IMPURITIES****Delete the following:**• **HEAVY METALS** (231)

Test preparation: 1.0 g in 25 mL of water

Acceptance criteria: NMT 20 ppm • (Official 1-Jan-2018)

**SPECIFIC TESTS**• **ALKALINITY**

**Sample:** 1.0 g

**Analysis:** Dissolve the Sample in 15 mL of carbon dioxide-free water in a small flask. As soon as the solution is complete, add 1.0 mL of 0.10 N hydrochloric acid, then add 0.05 mL of phenolphthalein TS.

Acceptance criteria: No pink color is produced within 5 s.

• **LOSS ON DRYING** (731)

**Analysis:** Dry at 105° for 3 h.

Acceptance criteria: NMT 5.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP β-Alanine RS

USP Calcium Pantothenate RS

USP Pantolactone RS

USP Sodium D-Pantoate RS

**Calcium Pantothenate Tablets****DEFINITION**

Calcium Pantothenate Tablets contain NLT 95.0% and NMT 115.0% of the labeled amount of the dextrorotatory isomer of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ).

**IDENTIFICATION**• **A. IDENTIFICATION TESTS—GENERAL**, Calcium (191)

**Sample solution:** Digest a quantity of powdered Tablets, equivalent to 150 mg of calcium pantothenate, with 15 mL of 1 N sodium hydroxide, and filter.

Acceptance criteria: Meet the requirements

• **B.**

**Sample solution:** 5 mL of the filtrate obtained in Identification test A

**Analysis:** Add 5 mL of 1 N hydrochloric acid and 2 drops of ferric chloride TS to the Sample solution.

Acceptance criteria: A strong yellow color is produced.

**ASSAY**• **CALCIUM PANTOTHENATE**

**Buffer solution:** Dissolve 10.0 g of monobasic potassium phosphate in 2000 mL of water, and adjust with phosphoric acid to a pH of 3.5.

**Mobile phase:** Methanol and Buffer solution (1:9)

**System suitability solution:** 0.5 mg/mL of USP Calcium Pantothenate RS and 0.1 mg/mL of USP Racemic Panthenol RS in water

**Standard solution:** 0.5 mg/mL of USP Calcium Pantothenate RS in water



**Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 50 mg of calcium pantothenate, to a 100-mL volumetric flask. Add 5 mL of methanol, and swirl the flask to disperse. Dilute with water to volume, mix, and filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 205 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Column temperature:** 50°

**Flow rate:** 2 mL/min

**Injection volume:** 25 μL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for pantothenate and panthenol are 1.0 and 1.1, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between pantothenate and panthenol

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Measure the peak areas for calcium pantothenate.

Calculate the percentage of the labeled amount of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Calcium Pantothenate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of calcium pantothenate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–115.0%

### OTHER COMPONENTS

#### • CONTENT OF CALCIUM

**Sample:** A portion of the powder from NLT 20 finely powdered Tablets, equivalent to 500 mg of calcium pantothenate

**Blank:** Proceed as directed in the *Analysis*, without the *Sample*.

#### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.05 M edetate disodium VS

**Indicator:** Hydroxy naphthol blue, 300 mg

**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a suitable crucible. Ignite, gently at first, until free from carbon. Cool the crucible. Add 10 mL of water, and dissolve the residue by adding sufficient 3 N hydrochloric acid, dropwise, to completely dissolve. Transfer the solution to a suitable container, and dilute with water to 150 mL. Add 15 mL of 1 N sodium hydroxide, then add the *Indicator*. Titrate with *Titrant* to a distinct blue endpoint. Perform a blank determination.

Calculate the percentage of calcium in the content of calcium pantothenate, as determined by the *Assay*, in the portion of Tablets taken:

$$\text{Result} = \{[(V_S - V_B) \times M \times F]/W\} \times 100$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$M$  = actual molarity of the *Titrant* (mM/mL)

$F$  = equivalency factor, 40.08 mg/mM

$W$  = weight of calcium pantothenate in the *Sample* taken, as determined by the *Assay* (mg)

**Acceptance criteria:** 7.9%–9.7% of the weight of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ) in the Tablets, as determined by the *Assay*

### PERFORMANCE TESTS

#### • DISSOLUTION, Procedure for a Pooled Sample (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Mobile phase:** Phosphoric acid and water (1:1000)

**Standard solution:** A known concentration of USP Calcium Pantothenate RS in *Medium*

**Sample solution:** A filtered portion of the solution under test, suitably diluted with *Medium* if necessary, having a concentration of  $C_{18}H_{32}CaN_2O_{10}$  similar to that of the *Standard solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 3.9-mm × 15-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 3.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times [C_S \times D \times (V/L)] \times 100$$

$r_U$  = peak area of calcium pantothenate from the *Sample solution*

$r_S$  = peak area of calcium pantothenate from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$D$  = dilution factor for the *Sample solution*

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of calcium pantothenate ( $C_{18}H_{32}CaN_2O_{10}$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE:

Preserve in tight containers.

#### • LABELING:

Label the Tablets to indicate the content of dextrorotatory calcium pantothenate.

#### • USP REFERENCE STANDARDS (11)

USP Calcium Pantothenate RS

USP Racemic Panthenol RS

## Racemic Calcium Pantothenate

$C_{18}H_{32}CaN_2O_{10}$  476.53

$\beta$ -Alanine, *N*-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-, calcium salt (2:1), (±)-;

Calcium DL-pantothenate (1:2) [6381-63-1].

### DEFINITION

Racemic Calcium Pantothenate is a mixture of the calcium salts of the dextrorotatory and levorotatory isomers of pantothenic acid. It contains NLT 5.7% and NMT 6.0% of nitrogen (N), and NLT 8.2% and NMT 8.6% of calcium (Ca), both calculated on the dried basis.

[NOTE—The physiological activity of Racemic Calcium Pantothenate is approximately one-half that of Calcium Pantothenate.]



**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B. IDENTIFICATION TESTS—GENERAL, Calcium (191):** A 50-mg/mL solution meets the requirements.

**COMPOSITION**

- **NITROGEN DETERMINATION, Method I (461)**  
Sample: 500 mg  
Analysis: Proceed as directed in the chapter.  
Acceptance criteria: 5.7%–6.0% on the dried basis
- **CONTENT OF CALCIUM**  
Sample: 800 mg  
Blank: 150 mL of water containing 2 mL of 3 N hydrochloric acid  
**Titrimetric system**  
(See *Titrimetry* (541).)  
Mode: Direct titration  
Titrant: 0.05 M edetate disodium VS  
Endpoint detection: Visual  
Analysis: Dissolve the *Sample* in 150 mL of water containing 2 mL of 3 N hydrochloric acid. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue, and titrate with the *Titrant* to a distinct blue endpoint. Perform the *Blank* determination. Calculate the percentage of calcium (Ca) in the *Sample* taken:  
$$\text{Result} = \frac{(V_S - V_B) \times M \times F}{W} \times 100$$
  
$$\begin{aligned} V_S &= \text{Titrant volume consumed by the Sample (mL)} \\ V_B &= \text{Titrant volume consumed by the Blank (mL)} \\ M &= \text{actual molarity of the Titrant (mM/mL)} \\ F &= \text{equivalency factor, 40.08 mg/mM} \\ W &= \text{Sample weight (mg)} \end{aligned}$$
  
Acceptance criteria: 8.2%–8.6% on the dried basis

**IMPURITIES****Delete the following:**

- **HEAVY METALS (231)**  
Test preparation: 1.0 g in 25 mL of water  
Acceptance criteria: NMT 20 ppm (Official 1-Jan-2018)

**SPECIFIC TESTS**

- **OPTICAL ROTATION, Specific Rotation (781S)**  
Test solution: 50 mg/mL in water  
Acceptance criteria:  $-0.05^\circ$  to  $+0.05^\circ$
- **ALKALINITY**  
Sample: 1.0 g  
Analysis: Dissolve the *Sample* in 15 mL of carbon dioxide-free water in a small flask. As soon as the solution is complete, add 1.6 mL of 0.10 N hydrochloric acid, and then add 0.05 mL of phenolphthalein TS.  
Acceptance criteria: No pink color is produced within 5 s.
- **LOSS ON DRYING (731):** Dry a sample at  $105^\circ$  for 3 h: it loses NMT 5.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label preparations containing it in terms of the equivalent amount of dextrorotatory calcium pantothenate.

- **USP REFERENCE STANDARDS (11)**  
USP Calcium Pantothenate RS

**Dibasic Calcium Phosphate Dihydrate****Pharmacopeial Discussion Group Sign-Off Document**

Attribute	JP	EP	USP
Definition	+	+	+
Identification A	+	+	+
Identification B	+	+	+
Acid-Insoluble Substances	+	+	+
Chloride	+	+	+
Sulfate	+	+	+
Carbonate	+	+	+
Barium	+	+	+
Loss on Ignition	+	+	+
Assay	+	+	+

**Legend:** +, will adopt and implement; –, will not stipulate.

**Change to read:**

**Nonharmonized attributes:** *Packaging and Storage*,  
• (Official 1-Jan-2018) *Limit of Fluoride, Iron*

**Specific local attributes:** Identification C (EP), Lead (USP), Description (JP)

$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  172.09  
Phosphoric acid, calcium salt (1:1);  
Calcium phosphate, dihydrate (1:1) [7789-77-7].

**DEFINITION**

Dibasic Calcium Phosphate Dihydrate contains two molecules of water of hydration. It contains NLT 98.0% and NMT 105.0% of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ).

**IDENTIFICATION**

- **A.**  
Sample: 0.1 g of Dibasic Calcium Phosphate Dihydrate  
Analysis: Dissolve the *Sample* by warming in 10 mL of 2 N hydrochloric acid. Add 2.5 mL of ammonia TS dropwise, with shaking, and then add 5 mL of ammonium oxalate TS.  
Acceptance criteria: A white precipitate is formed.
- **B.**  
Sample: 0.1 g of Dibasic Calcium Phosphate Dihydrate  
Analysis: Dissolve the *Sample* in 5 mL of diluted nitric acid. Warm the solution to  $70^\circ$ , and add 2 mL of 10% ammonium molybdate solution (freshly prepared).  
Acceptance criteria: A yellow precipitate of ammonium phosphomolybdate is formed.

**ASSAY**

- **PROCEDURE**  
Buffer: Dissolve 53.5 g of ammonium chloride with sufficient water in a 1000-mL volumetric flask. Add 570 mL of ammonia water, stronger, and dilute with water to volume. The pH of this solution is 10.7.  
Sample solution: Transfer 400 mg of Dibasic Calcium Phosphate Dihydrate to a 200-mL volumetric flask. Dissolve in 12 mL of diluted hydrochloric acid with the aid of gentle heat, if necessary, and dilute with water to volume.  
Blank: 20 mL of water containing 1.2 mL of diluted hydrochloric acid



**Titrimetric system**(See *Titrimetry* (541).)**Mode:** Residual titration**Titrant:** 0.02 M edetate disodium VS**Back-titrant:** 0.02 M zinc sulfate VS**Endpoint detection:** Visual

**Analysis:** To 20.0 mL of the *Sample solution* add 25.0 mL of *Titrant*, 50 mL of water, and 5 mL of *Buffer*. Add 25 mg of eriochrome black T–sodium chloride indicator. Titrate the excess *Titrant* with the *Back-titrant*. Perform a *Blank* determination in the same manner. Calculate the percentage of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) in the sample taken:

$$\text{Result} = \{[(V_B - V_S) \times M \times F]/W\} \times 100$$

$V_B$  = *Back-titrant* volume consumed by the *Blank* (mL)

$V_S$  = *Back-titrant* volume consumed by the *Sample* (mL)

$M$  = actual molarity of the *Back-titrant* (mM/mL)

$F$  = equivalency factor, 172.1 mg/mM

$W$  = *Sample weight* (mg) in 20.0 mL of the *Sample solution*

**Acceptance criteria:** 98.0%–105.0%

**IMPURITIES****• CARBONATE**

**Sample:** 1.0 g of Dibasic Calcium Phosphate Dihydrate  
**Analysis:** Mix the *Sample* with 5 mL of carbon dioxide-free water, and immediately add 2 mL of hydrochloric acid.

**Acceptance criteria:** No effervescence occurs.

**• CHLORIDE AND SULFATE, Chloride (221)**

**Standard:** 0.70 mL of 0.010 N hydrochloric acid

**Sample:** 0.2 g of Dibasic Calcium Phosphate Dihydrate

**Analysis:** To the *Sample* add 20 mL of water and 13 mL of diluted nitric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to 100 mL, and filter if necessary. To 50 mL of the filtrate add 1 mL of silver nitrate TS.

**Acceptance criteria:** The turbidity of the *Sample* does not exceed that of the *Standard* (NMT 0.25%).

**• CHLORIDE AND SULFATE, Sulfate (221)**

**Standard:** 1.0 mL of 0.010 N sulfuric acid

**Sample:** 0.5 g of Dibasic Calcium Phosphate Dihydrate

**Analysis:** To the *Sample* add 5 mL of water and 5 mL of diluted hydrochloric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to 100 mL, and filter if necessary. To 20 mL of the filtrate add 1 mL of diluted hydrochloric acid, and dilute with water to 50 mL. Add 1 mL of barium chloride TS.

**Acceptance criteria:** The turbidity of the *Sample* does not exceed that of the *Standard* (NMT 0.5%).

**• ARSENIC, Method I (211)**

**Test preparation:** 1.0 g in 25 mL of 3 N hydrochloric acid, diluted with water to 55 mL. Omit the addition of 20 mL of 7 N sulfuric acid specified in *Procedure*.

**Acceptance criteria:** NMT 3 µg/g

**• BARIUM**

**Sample:** 0.5 g Dibasic Calcium Phosphate Dihydrate

**Analysis:** Heat the *Sample* to boiling with 10 mL of water, and add 1 mL of hydrochloric acid dropwise, stirring after each addition. Allow to cool, and filter, if necessary. To the filtrate add 2 mL of potassium sulfate TS.

**Acceptance criteria:** No turbidity is produced within 10 min.

**Delete the following:****• HEAVY METALS, Method I (231)**

**Test preparation:** Warm 1.3 g with 3 mL of 3 N hydrochloric acid until no more dissolves. Cool, dilute with water to 50 mL, and filter.

**Acceptance criteria:** NMT 30 ppm (Official 1-Jan-2018)

**• LIMIT OF ACID-INSOLUBLE SUBSTANCES**

**Sample solution:** Dissolve 5.0 g in a mixture of 40 mL of water and 10 mL of hydrochloric acid by boiling gently for 5 min.

**Analysis:** After cooling, collect the insoluble substance on ashless filter paper, and wash with water until the last washing does not give a reaction for chloride (no turbidity results from the addition of silver nitrate TS). Ignite to completely incinerate the residue and the ashless filter paper at  $600 \pm 50^\circ$ .

**Acceptance criteria:** The weight of the residue does not exceed 10 mg (NMT 0.2%).

**• LIMIT OF FLUORIDE**

[NOTE—Prepare and store all solutions in plastic containers.]

**Buffer solution:** 294 mg/mL of sodium citrate dihydrate in water

**Standard stock solution:** 1.1052 mg/mL of USP Sodium Fluoride RS in water

**Standard solution:** Transfer 20.0 mL of *Standard stock solution* to a 100-mL volumetric flask containing 50.0 mL of *Buffer solution*, dilute with water to volume, and mix. Each mL of this solution contains 100 µg of fluoride ion.

**Sample solution:** Transfer 2.0 g of Dibasic Calcium Phosphate dihydrate to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of *Buffer solution* and sufficient water to make 100 mL.

**Electrode system:** Use a fluoride-specific ion-indicating electrode and a silver–silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of  $\pm 0.2$  mV (see pH (791)).

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Standard response line:** Transfer 50.0 mL of *Buffer solution* and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500 µL of *Standard solution*, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0 µg/mL) versus potential in mV.

Rinse and dry the electrodes, insert them into the *Sample solution*, stir for 5 min, and read the potential in mV. From the measured potential and the *Standard response line* determine the concentration,  $C$  (in µg/mL), of fluoride ion in the *Sample solution*.

Calculate the content of fluoride (ppm) in the portion of Dibasic Calcium Phosphate Dihydrate taken:

$$\text{Result} = (V \times C)/W$$

$V$  = *Sample solution* volume (mL)

$C$  = concentration of fluoride ion, determined from the *Standard response line*, in the *Sample solution* (µg/mL)

$W$  = weight of Dibasic Calcium Phosphate Dihydrate taken to prepare the *Sample solution* (g)

**Acceptance criteria:** NMT 50 ppm



**SPECIFIC TESTS**• **LOSS ON IGNITION** (733)

**Sample:** 1 g of Dibasic Calcium Phosphate Dihydrate  
**Analysis:** Ignite the *Sample* at 800°–825° to constant weight.

**Acceptance criteria:** 24.5%–26.5%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.

• **USP REFERENCE STANDARDS** (11)

USP Sodium Fluoride RS

**Anhydrous Dibasic Calcium Phosphate****Pharmacopeial Discussion Group Sign-Off Document**

Attribute	JP	EP	USP
Definition	+	+	+
Identification A	+	+	+
Identification B	+	+	+
Acid-insoluble substances	+	+	+
Chloride	+	+	+
Sulfate	+	+	+
Carbonate	+	+	+
Barium	+	+	+
Loss on ignition	+	+	+
Assay	+	+	+

**Legend:** + will adopt and implement; - will not stipulate.

**Change to read:**

**Nonharmonized attributes:** *Packaging and storage,*

• (Official: 1-Jan-2018) *Limit of fluoride, Iron*

**Specific local attributes:** Identification C (EP), Lead (USP), Description (JP)

CaHPO<sub>4</sub> 136.06  
 Phosphoric acid, calcium salt (1:1);  
 Calcium phosphate (1:1) [7757-93-9].

**DEFINITION**

Anhydrous Dibasic Calcium Phosphate contains NLT 98.0% and NMT 103.0% of anhydrous dibasic calcium phosphate (CaHPO<sub>4</sub>).

**IDENTIFICATION**

- **A.**  
**Sample:** 0.1 g of Anhydrous Dibasic Calcium Phosphate  
**Analysis:** Dissolve the *Sample* by warming in 10 mL of 2 N hydrochloric acid. Add 2.5 mL of ammonia TS dropwise, with shaking, and then add 5 mL of ammonium oxalate TS.  
**Acceptance criteria:** A white precipitate is formed.
- **B.**  
**Sample:** 0.1 g of Anhydrous Dibasic Calcium Phosphate  
**Analysis:** Dissolve the *Sample* in 5 mL of diluted nitric acid. Warm the solution to 70°, and add 2 mL of 10% ammonium molybdate solution (freshly prepared).  
**Acceptance criteria:** A yellow precipitate of ammonium phosphomolybdate is formed.

**ASSAY**• **PROCEDURE**

**Buffer:** Dissolve 53.5 g of ammonium chloride with sufficient water in a 1000-mL volumetric flask. Add 570 mL of ammonia water, stronger, and dilute with water to volume. The pH of this solution is 10.7.

**Sample solution:** Transfer 400 mg of Anhydrous Dibasic Calcium Phosphate to a 200-mL volumetric flask. Dissolve in 12 mL of diluted hydrochloric acid with the aid of gentle heat, if necessary, and dilute with water to volume.

**Blank:** 20 mL of water containing 1.2 mL of diluted hydrochloric acid

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Residual titration

**Titrant:** 0.02 M edetate disodium VS

**Back-titrant:** 0.02 M zinc sulfate VS

**Endpoint detection:** Visual

**Analysis:** To 20.0 mL of the *Sample solution* add 25.0 mL of *Titrant*, 50 mL of water, and 5 mL of *Buffer*. Add 25 mg of eriochrome black T-sodium chloride indicator. Titrate the excess *Titrant* with the *Back-titrant*. Perform a *Blank* determination in the same manner. Calculate the percentage of anhydrous dibasic calcium phosphate (CaHPO<sub>4</sub>) in the sample taken:

$$\text{Result} = \{[(V_B - V_S) \times M \times F] / W\} \times 100$$

$V_B$  = *Back-titrant* volume consumed by the *Blank* (mL)

$V_S$  = *Back-titrant* volume consumed by the *Sample* (mL)

$M$  = actual molarity of the *Back-titrant* (mM/mL)

$F$  = equivalency factor, 136.06 mg/mM

$W$  = *Sample weight* (mg) in 20.0 mL of the *Sample solution*

**Acceptance criteria:** 98.0%–103.0%

**IMPURITIES**• **CARBONATE**

**Sample:** 1.0 g of Anhydrous Dibasic Calcium Phosphate

**Analysis:** Mix the *Sample* with 5 mL of carbon dioxide-free water, and immediately add 2 mL of hydrochloric acid.

**Acceptance criteria:** No effervescence occurs.

• **CHLORIDE AND SULFATE, Chloride** (221)

**Standard:** 0.70 mL of 0.010 N hydrochloric acid

**Sample:** 0.2 g of Anhydrous Dibasic Calcium Phosphate

**Analysis:** To the *Sample* add 20 mL of water and 13 mL of diluted nitric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to 100 mL, and filter if necessary. To 50 mL of the filtrate add 1 mL of silver nitrate TS.

**Acceptance criteria:** The turbidity of the *Sample* does not exceed that of the *Standard* (NMT 0.25%).

• **CHLORIDE AND SULFATE, Sulfate** (221)

**Standard:** 1.0 mL of 0.010 N sulfuric acid

**Sample:** 0.5 g of Anhydrous Dibasic Calcium Phosphate

**Analysis:** To the *Sample* add 5 mL of water and 5 mL of diluted hydrochloric acid, and warm gently, if necessary, to completely dissolve. Dilute with water to 100 mL, and filter if necessary. To 20 mL of the filtrate add 1 mL of diluted hydrochloric acid, and dilute with water to 50 mL. Add 1 mL of barium chloride TS.

**Acceptance criteria:** The turbidity of the *Sample* does not exceed that of the *Standard* (NMT 0.5%).

• **ARSENIC, Method I** (211)

**Test preparation:** 1.0 g in 25 mL of 3 N hydrochloric acid, diluted with water to 55 mL. Omit the addition of 20 mL of 7 N sulfuric acid specified in *Procedure*.

**Acceptance criteria:** NMT 3 µg/g

• **BARIUM**

**Sample:** 0.5 g Anhydrous Dibasic Calcium Phosphate

**Analysis:** Heat the *Sample* to boiling with 10 mL of water, and add 1 mL of hydrochloric acid dropwise, stirring after each addition. Allow to cool, and filter if necessary. To the filtrate add 2 mL of potassium sulfate TS.  
**Acceptance criteria:** No turbidity is produced within 10 min.



**Delete the following:**

- **HEAVY METALS, Method I (231)**  
Test preparation: Warm 1.3 g with 3 mL of 3 N hydrochloric acid until no more dissolves. Cool, dilute with water to 50 mL, and filter.  
Acceptance criteria: NMT 30 ppm (Official 1-Jan-2018)
- **LIMIT OF ACID-INSOLUBLE SUBSTANCES**  
Sample solution: Dissolve 5.0 g in a mixture of 40 mL of water and 10 mL of hydrochloric acid by boiling gently for 5 min.  
Analysis: After cooling, collect the insoluble substance on ashless filter paper, and wash with water until the last washing does not give a reaction for chloride (no turbidity results from the addition of silver nitrate TS). Ignite to completely incinerate the residue and the ashless filter paper at  $600 \pm 50^\circ$ .  
Acceptance criteria: The weight of the residue does not exceed 10 mg (NMT 0.2%).
- **LIMIT OF FLUORIDE**  
[NOTE—Prepare and store all solutions in plastic containers.]  
Buffer solution: 294 mg/mL of sodium citrate dihydrate in water  
Standard stock solution: 1.1052 mg/mL of USP Sodium Fluoride RS in water  
Standard solution: Transfer 20.0 mL of Standard stock solution to a 100-mL volumetric flask containing 50.0 mL of Buffer solution, dilute with water to volume, and mix. Each mL of this solution contains 100  $\mu$ g of fluoride ion.  
Sample solution: Transfer 2.0 g of Anhydrous Dibasic Calcium Phosphate to a beaker containing a plastic-coated stirring bar. Add 20 mL of water and 2.0 mL of hydrochloric acid, and stir until dissolved. Add 50.0 mL of Buffer solution and sufficient water to make 100 mL.  
Electrode system: Use a fluoride-specific ion-indicating electrode and a silver-silver chloride reference electrode connected to a pH meter capable of measuring potentials with a minimum reproducibility of  $\pm 0.2$  mV (see pH (791)).  
Analysis  
Samples: Standard solution and Sample solution  
Standard response line: Transfer 50.0 mL of Buffer solution and 2.0 mL of hydrochloric acid to a beaker, and add water to make 100 mL. Add a plastic-coated stirring bar, insert the electrodes into the solution, stir for 15 min, and read the potential in mV. Continue stirring, and at 5-min intervals add 100, 100, 300, and 500  $\mu$ L of Standard solution, reading the potential 5 min after each addition. Plot the logarithms of the cumulative fluoride ion concentrations (0.1, 0.2, 0.5, and 1.0  $\mu$ g/mL) versus potential in mV.  
Rinse and dry the electrodes, insert them into the Sample solution, stir for 5 min, and read the potential in mV. From the measured potential and the Standard response line determine the concentration, C (in  $\mu$ g/mL), of fluoride ion in the Sample solution.  
Calculate the content of fluoride (ppm) in the portion of Anhydrous Dibasic Calcium Phosphate taken:

$$\text{Result} = (V \times C)/W$$

- V = Sample solution volume (mL)  
C = concentration of fluoride ion, determined from the Standard response line, in the Sample solution ( $\mu$ g/mL)  
W = weight of Anhydrous Dibasic Calcium Phosphate taken to prepare the Sample solution (g)

Acceptance criteria: NMT 50 ppm

**SPECIFIC TESTS**

- **LOSS ON IGNITION (733)**  
Sample: 1 g of Anhydrous Dibasic Calcium Phosphate  
Analysis: Ignite the Sample at  $800^\circ$ – $825^\circ$  to constant weight.  
Acceptance criteria: 6.6%–8.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. No storage requirements specified.
- **USP REFERENCE STANDARDS (11)**  
USP Sodium Fluoride RS

**Dibasic Calcium Phosphate Tablets****DEFINITION**

Dibasic Calcium Phosphate Tablets contain NLT 92.5% and NMT 107.5% of the labeled amount of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ).

[NOTE—An equivalent amount of Dibasic Calcium Phosphate with less water of hydration may be used in place of  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  in preparing Dibasic Calcium Phosphate Tablets.]

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191)**  
Sample solution: A filtered portion of the Sample solution from the Assay  
Acceptance criteria: Meets the requirements
- **B. IDENTIFICATION TESTS—GENERAL, Phosphate (191)**  
Sample solution: A filtered portion of the Sample solution from the Assay, neutralized with ammonium hydroxide  
Acceptance criteria: Meets the requirements

**ASSAY****• PROCEDURE**

Sample solution: Transfer a portion of powder, equivalent to 1 g of dibasic calcium phosphate dihydrate, from NLT 20 powdered Tablets, to a 100-mL volumetric flask containing 15 mL of hydrochloric acid and 10 mL of water. Heat on a steam bath, with occasional mixing, to dissolve the dibasic calcium phosphate, but not longer than 30 min. Cool, add water to volume, and mix. If the solution is not clear, filter, discarding the first 10 mL of the filtrate.

Blank: Water

Titrimetric system

(See Titrimetry (541).)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Indicator: Hydroxy naphthol blue

Endpoint detection: Visual

Analysis: Transfer 25.0 mL of the Sample solution to a 250-mL beaker equipped with a magnetic stirrer. With constant stirring, add, in the order named, 0.5 mL of triethanolamine, 300 mg of Indicator, and from a 50-mL buret, about 23 mL of Titrant. Add sodium hydroxide solution (45 in 100) until the initial red color changes to clear blue. Continue to add it dropwise until the color changes to violet, and add an additional 0.5 mL. The pH is 12.3–12.5. Continue the titration dropwise with the Titrant to the appearance of a clear blue endpoint that persists for NLT 60 s. Perform a blank determination.

Calculate the percentage of the labeled amount of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) in the portion of Tablets taken:

$$\text{Result} = [(V_s - V_0) \times N \times F]/W \times 100$$



- $V_S$  = Titrant volume consumed by the *Sample solution* (mL)  
 $V_B$  = Titrant volume consumed by the *Blank* (mL)  
 $M$  = actual molarity of the *Titrant* (mM/mL)  
 $F$  = equivalency factor, 172.08 mg/mM  
 $W$  = nominal weight of dibasic calcium phosphate dihydrate in the *Sample solution* taken for Analysis (mg)

Acceptance criteria: 92.5%–107.5%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 900 mL

Apparatus 2: 75 rpm

Time: 45 min

Standard solution: Solution having a known concentration of calcium in *Medium*

Sample solution: Filtered portion of the solution under test, suitably diluted with *Medium* if necessary

### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 422.7 nm

Lamp: Calcium hollow-cathode

Flame: Air-acetylene

### Analysis

Samples: *Standard solution* and *Sample solution*

Determine the concentration of calcium (Ca) in the *Sample solution* in comparison with a *Standard solution*.

Calculate the percentage of the labeled amount of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S \times D \times V/L) \times (M_r/A_r) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of calcium in the *Standard solution* (mg/mL)

$D$  = dilution factor for the *Sample solution*

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

$M_r$  = molecular weight of dibasic calcium phosphate, 172.08

$A_r$  = atomic weight of calcium, 40.08

Tolerances: NLT 75% (Q) of the labeled amount of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** The quantity of dibasic calcium phosphate stated in the labeling is in terms of dibasic calcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ).

## Calcium Polycarbophil

Calcium polycarbophil [9003-97-8].

» Calcium Polycarbophil is the calcium salt of polyacrylic acid cross-linked with divinyl glycol.

**Packaging and storage**—Preserve in tight containers.

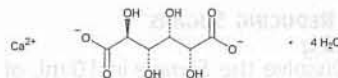
**Identification**—When tested as directed in the test for *Absorbing power*, it absorbs about 35 times its original weight.

**Loss on drying** (731)—Dry it in vacuum at 130° for 4 hours: it loses not more than 10.0% of its weight.

**Absorbing power**—Transfer about 250 mg, accurately weighed, to a tared 50-mL centrifuge tube fitted with a tight closure. Add 35 mL of 0.1 N hydrochloric acid to the tube, seal the tube, and shake by mechanical means for 30 minutes. Centrifuge at 2000 rpm for 20 minutes, and decant and discard the supernatant. [NOTE—Exercise care to avoid any loss of particles.] Add 35 mL of 0.1 N hydrochloric acid, and shake for 30 minutes. Centrifuge, decanting and discarding the supernatant. Repeat the foregoing steps, using water instead of acid. Add 35 mL of a sodium bicarbonate solution (15 in 1000), and shake, venting as necessary to release any carbon dioxide liberated. Shake for 1 hour, centrifuge, and decant the supernatant. Add 35 mL of sodium bicarbonate solution (15 in 1000), and shake for 1 hour. Allow the tube and contents to stand overnight or until the contents have settled, and centrifuge. Withdraw the supernatant, and weigh the tube and contents. Calculate the weight of sodium bicarbonate solution absorbed: not less than 35.0 g of the sodium bicarbonate solution is absorbed by 1.0 g of Calcium Polycarbophil, calculated on the dried basis.

**Content of calcium**—Transfer about 2 g of Calcium Polycarbophil, accurately weighed, to a tared crucible. Cover, leaving the lid slightly ajar, and place in a muffle furnace. Heat to 600° over 2 hours, increase the temperature to 1000° over 1 hour, and maintain at 1000° for 1 hour. Allow to cool slowly. Dissolve the residue in dilute hydrochloric acid (1 in 5), quantitatively transfer with the aid of dilute hydrochloric acid (1 in 5) to a 100-mL volumetric flask, and dilute with dilute hydrochloric acid (1 in 5) to volume. Pipet 15 mL of this solution into a 250-mL beaker, and add, while stirring with a magnetic stirrer, 100 mL of water, 20.0 mL of 0.05 M edetate disodium VS, and 300 mg of hydroxy naphthol blue. Adjust with 1 N sodium hydroxide solution to a pH of 9.0 to 9.5. Adjust with about 10 mL of 2 N sodium hydroxide to a pH of 12.4. Titrate with 0.05 M edetate disodium VS to a persistent blue endpoint. Each mL of 0.05 M edetate disodium is equivalent to 2.004 mg of calcium (Ca). The content of Ca found is not less than 18.0% and not more than 22.0%, calculated on the dried basis.

## Calcium Saccharate



$\text{C}_6\text{H}_8\text{CaO}_8 \cdot 4\text{H}_2\text{O}$  320.26  
 D-Glucaric acid, calcium salt (1:1) tetrahydrate;  
 Calcium D-glucarate (1:1), tetrahydrate [5793-89-5].

## DEFINITION

Calcium Saccharate is the calcium salt of D-saccharic acid. It contains NLT 98.5% and NMT 102.0% of  $\text{C}_6\text{H}_8\text{CaO}_8 \cdot 4\text{H}_2\text{O}$ .

## IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium** (191)

Sample: 0.2 g

Analysis: Dissolve the *Sample* in 10 mL of water by the addition of 2 mL of hydrochloric acid.



Acceptance criteria: Meets the requirements

- **B. INFRARED ABSORPTION (197M):** Meets the requirements

## ASSAY

### • PROCEDURE

Sample: 600 mg

Blank: 150.0 mL of water

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.05 M edetate disodium VS

Endpoint detection: Visual

Analysis: Dissolve the *Sample* in 150 mL of water with the aid of a sufficient volume of hydrochloric acid.

While stirring, preferably with a magnetic stirrer, add 30 mL of *Titrant* from a 50-mL buret. Add 15 mL of 1 N sodium hydroxide and 300 mg of hydroxy naphthol blue. Continue the titration to a blue endpoint. Perform a *Blank* determination and make any necessary corrections.

Calculate the percentage of calcium saccharate ( $C_6H_8CaO_8 \cdot 4H_2O$ ) in the portion of Calcium Saccharate taken:

$$\text{Result} = \frac{[(V_S - V_B) \times N \times F]/W}{100}$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the *Blank* (mL)

$N$  = actual normality of the *Titrant* (mEq/mL)

$F$  = equivalency factor, 320.2 mg/mEq

$W$  = *Sample* weight (mg)

Acceptance criteria: 98.5%–102.0%

## IMPURITIES

- **CHLORIDE AND SULFATE, Chloride (221)**

Standard: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.50 g dissolved in 10 mL of water by the addition of 2 mL of nitric acid

Acceptance criteria: NMT 0.07%

- **CHLORIDE AND SULFATE, Sulfate (221)**

Standard: 0.6 mL of 0.020 N sulfuric acid

Sample: 0.50 g dissolved in 10 mL of water by the addition of 2 mL of hydrochloric acid

Acceptance criteria: NMT 0.12%

## Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1, Jan-2018)

- **SUCROSE AND REDUCING SUGARS**

Sample: 0.5 g

Analysis: Dissolve the *Sample* in 10 mL of water with the addition of 2 mL of hydrochloric acid, and boil the solution for about 2 min. Cool, add 15 mL of sodium carbonate TS, allow to stand for 5 min, and filter. Add 5 mL of the clear filtrate to about 2 mL of alkaline cupric tartrate TS, and boil for 1 min.

Acceptance criteria: No red precipitate is formed immediately.

## SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 60 mg/mL in 4.8 N hydrochloric acid that has been allowed to stand for 1 h

Acceptance criteria: +18.5° to +22.5°

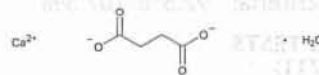
## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**

USP Calcium Saccharate RS

## Calcium Succinate



$C_4H_4CaO_4 \cdot H_2O$

174.17

Butanedioic acid, calcium salt (1:1), monohydrate;

Calcium succinate monohydrate [159389-75-0].

Anhydrous [140-99-8].

## DEFINITION

Calcium Succinate contains NLT 98.0% and NMT 102.0% of calcium succinate ( $C_4H_4CaO_4$ ), calculated on the dried basis.

## IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191)**

Sample solution: 20 mg/mL

Acceptance criteria: Meets the requirements

- **B. INFRARED ABSORPTION (197K)**

## ASSAY

### • PROCEDURE

Sample: 650 mg of Calcium Succinate

Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 M edetate disodium VS

Endpoint detection: Visual

Analysis: Transfer the *Sample* into a 250-mL beaker, add 100 mL of water and 2 mL of concentrated hydrochloric acid, and stir using a magnetic stirring bar.

While stirring, add 30 mL of *Titrant* from the titration buret, and 25 mL of 2 N sodium hydroxide. Check the pH of the solution and make sure it is between 12.0 and 13.0. Add 300–500 mg of hydroxy naphthol blue, and continue the titration to a blue endpoint. Perform a blank determination.

Calculate the percentage of calcium succinate ( $C_4H_4CaO_4$ ) in the *Sample* taken:

$$\text{Result} = \frac{[(V_S - V_B) \times M \times F]/W}{100}$$

$V_S$  = *Titrant* volume consumed by the *Sample* (mL)

$V_B$  = *Titrant* volume consumed by the blank (mL)

$M$  = actual molarity of the *Titrant* (mmol/mL)

$F$  = equivalency factor, 156.15 mg/mmol

$W$  = *Sample* weight (mg)

Acceptance criteria: 98.0%–102.0% on the dried basis

## IMPURITIES

- **CHLORIDE AND SULFATE, Chloride (221)**

Standard solution: 1.0 mL of 0.010 N hydrochloric acid



- Sample: 1.8 g  
 Acceptance criteria: NMT 0.02%
- **CHLORIDE AND SULFATE, Sulfate (221)**  
 Standard solution: 1.0 mL of 0.020 N sulfuric acid  
 Sample: 2.0 g  
 Acceptance criteria: NMT 0.05%
  - **ELEMENTAL IMPURITIES—PROCEDURES (233)**  
 Acceptance criteria  
 Arsenic: NMT 3.0 µg/g  
 Cadmium: NMT 2.0 µg/g  
 Lead: NMT 2.0 µg/g  
 Mercury: NMT 0.1 µg/g

**SPECIFIC TESTS**

- **PH (791)**  
 Sample solution: 50 mg/mL in water. [NOTE—The solution is slurry.]  
 Acceptance criteria: 7.0–12.0
- **LOSS ON DRYING (731)**  
 Analysis: Dry at 160° for 5 h.  
 Acceptance criteria: 9.0%–13.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
 USP Calcium Succinate RS

## Calcium Undecylenate



$\text{C}_{22}\text{H}_{38}\text{O}_4\text{Ca}$  406.61  
 10-Undecenoic acid, calcium (2+) salt;  
 Calcium 10-undecenoate [1322-14-1].

**DEFINITION**

Calcium Undecylenate contains NLT 98.0% and NMT 102.0% of calcium undecylenate ( $\text{C}_{22}\text{H}_{38}\text{O}_4\text{Ca}$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Calcium (191)**  
 Sample solution: A filtered solution (1 in 20) of Calcium Undecylenate in 3 N hydrochloric acid  
 Acceptance criteria: Meets the requirements
- **B. MELTING RANGE OR TEMPERATURE, Class Ia (741)**  
 Sample solution: Suspend 10 g of Calcium Undecylenate in 40 mL of water in a 250-mL separator. Cautiously and slowly add 10 mL of hydrochloric acid while swirling. Insert the stopper, and shake. The separator will become quite warm, and pressure must be carefully and frequently relieved through the stopcock. If a curdy, white material remains after 5 min of shaking, add additional hydrochloric acid, 1 mL at a time, and shake until a clear oily phase is formed.  
 Analysis: Allow the phases to separate, drain, and discard the bottom aqueous layer. Drain and discard the middle oily layer, if present. Filter the top layer of undecylenic acid through a pledget of cotton, noting the volume obtained. To the filtrate add an equal volume of aniline. Reflux for 1 h, swirling the flask occasionally. Allow to cool, and pour 60 mL of alcohol through the condenser into the flask. Remove the flask from the condenser, add 1 g of charcoal, and stir. Filter the slurry. Add water dropwise until a few crystals form or the solution becomes slightly cloudy. If too much water is added, an oil will form. Add alcohol dropwise until

the oil dissolves. Allow the mixture to stand or refrigerate until crystals are formed. Collect the crystals on a filter paper inserted in a 45-mm porous glass filter funnel. Wash the crystals with 75 mL of 25% alcohol: the crystals have a clean, white, glossy appearance. If not, recrystallize by dissolving the crystals in about 50 mL of alcohol. Add about 1 g of charcoal, stir, filter, and continue as directed above, beginning with "Add water dropwise". Dry the crystals under vacuum at 50° for 2 h. If the melting point is low, additional drying or recrystallization may be necessary.

Acceptance criteria: The crystals so obtained melt at 66°–67.5°.

**ASSAY**• **PROCEDURE**

**Sample solution:** Boil 40.0 mL of 0.1 N hydrochloric acid VS with 600 mg of Calcium Undecylenate for 10 min, or until the undecylenic acid layer is clear, adding water as necessary, to maintain the original volume. Transfer the mixture, with the aid of water, to a 500-mL separator. Dilute with water to about 75 mL, and extract with two 100-mL portions of solvent hexane. Wash the combined extracts with water until the last washing is neutral to litmus, and add the washings to the original water layer. Cool.

**Titrimetric system**

(See *Titrimetry (541)*, *Residual Titrations*.)

**Mode:** Residual titration

**Titrant:** 0.1 N hydrochloric acid VS

**Back-titrant:** 0.1 N sodium hydroxide VS

**Endpoint detection:** Visual

**Analysis:** Add 3 drops of methyl orange TS to the *Sample solution*. Then titrate the excess *Titrant* with *Back-titrant*. Perform a blank determination. Each mL of hydrochloric acid is equivalent to 20.33 mg of calcium undecylenate ( $\text{C}_{22}\text{H}_{38}\text{O}_4\text{Ca}$ ).

Acceptance criteria: 98.0%–102.0% on the dried basis

**IMPURITIES**• **LIMIT OF FREE UNDECYLENIC ACID**

**Sample solution:** Transfer 10 g of Calcium Undecylenate to a 400-mL beaker, add 250 mL of solvent hexane, and mix for 2 h using a magnetic stirrer. Filter into a 500-mL flask, and evaporate with the aid of a current of air to about 20 mL.

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N sodium hydroxide VS

**Endpoint detection:** Visual

**Analysis:** To the *Sample solution* add 100 mL of neutralized alcohol. Add 3 drops of phenolphthalein TS, and titrate with *Titrant*. Each mL of 0.1 N sodium hydroxide is equivalent to 18.43 mg of free undecylenic acid ( $\text{C}_{11}\text{H}_{20}\text{O}_2$ ).

Acceptance criteria: NMT 0.1%

**SPECIFIC TESTS**• **LOSS ON DRYING (731)**

**Analysis:** Dry at 105° for 2 h.

Acceptance criteria: 2.0%–5.7%

• **PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING (786)**

**Analysis:** Test in accordance with this procedure, except use NMT 25 g and use a single No. 100 sieve that is to be shaken for NLT 30 min or until sifting is practically complete.

Acceptance criteria: NLT 99.0% of it passes through a No. 100 sieve.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.



## Camphor



$C_{10}H_{16}O$  152.23  
Bicyclo[2.2.1]heptane-2-one, 1,7,7-trimethyl-;  
Camphor;  
2-Bornanone [76-22-2].

### DEFINITION

Camphor is a ketone of *Cinnamomum camphora* (L.) Nees et Ebermaier (Fam. Lauraceae) (natural Camphor), or is produced synthetically (synthetic Camphor).

### IMPURITIES

#### • LIMIT OF NONVOLATILE RESIDUE

Sample: 2.0 g of Camphor

Analysis: Heat the Sample in a tared dish on a steam bath until sublimation is complete. Dry the residue at 120° for 3 h, cool, and weigh.

Acceptance criteria: 0.05%; the weight of the residue does not exceed 1.0 mg.

#### • HALOGENS

Sample: Mix 100 mg of finely divided Camphor with 200 mg of sodium peroxide in a clean, dry, hard glass test tube about 25 mm in internal diameter and 20 cm in length. Suspend the tube at an angle of about 45°, using a clamp placed at the upper end. Gently heat the tube, starting near the upper end, but not heating the clamp. Gradually bring the heat toward the lower part of the tube until incineration is complete.

Analysis: Dissolve the residue in 25 mL of warm water, acidify with nitric acid, and filter the solution into a comparison tube. Wash the test tube and the filter with two 10-mL portions of hot water, adding the washings to the filtered solution. To the filtrate add 0.50 mL of 0.10 N silver nitrate, dilute with water to 50 mL, and mix.

Acceptance criteria: 0.035%; the turbidity does not exceed that produced in a blank test with the same quantities of the same reagents and 0.050 mL of 0.020 N hydrochloric acid.

### SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE** (741): 174°–179°

• **OPTICAL ROTATION, Specific Rotation** (781S)

Sample solution: 100 mg/mL in alcohol

Synthetic Camphor is optically inactive.

Acceptance criteria: +41° to +43° for natural Camphor

• **APPEARANCE OF SOLUTION:** A 100-mg/mL solution in solvent hexane is clear.

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.

• **LABELING:** Label it to indicate whether it is of natural sources or is prepared synthetically.

## Camphor Spirit

### DEFINITION

Camphor Spirit is an alcohol solution containing NLT 9.0 g and NMT 11.0 g of camphor ( $C_{10}H_{16}O$ ) in each 100 mL. Prepare Camphor Spirit as follows.

Camphor	100 g
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Alcohol, a sufficient quantity to make	1000 mL
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Dissolve the Camphor in 800 mL of the Alcohol, and add sufficient Alcohol to bring to final volume. Filter, if necessary.

### ASSAY

#### • PROCEDURE

Sample: 2.0 mL

Analysis: Transfer the Sample to a suitable pressure bottle containing 50 mL of freshly prepared dinitrophenylhydrazine TS. Close the pressure bottle, immerse it in a water bath, and maintain at about 75° for 16 h. Cool to room temperature, and transfer the contents to a beaker with the aid of 100 mL of 3 N sulfuric acid. Allow to stand at room temperature for NLT 12 h, transfer the precipitate to a tared filter crucible, and wash with 100 mL of 3 N sulfuric acid followed by 75 mL of cold water in divided portions. Continue the suction until the excess water is removed, dry the crucible and precipitate at 80° for 2 h, cool, and weigh. The weight of the precipitate so obtained, multiplied by 0.4581, represents the weight of camphor ( $C_{10}H_{16}O$ ) in the portion taken.

Acceptance criteria: 9.0–11.0 g of camphor in 100 mL

### OTHER COMPONENTS

#### • ALCOHOL DETERMINATION, Method II (611)

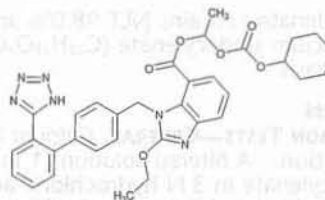
Test stock preparation: Dilute the Spirit with methanol to obtain a solution containing approximately 2% (v/v) of alcohol.

Acceptance criteria: 80.0%–87.0% of alcohol ( $C_2H_5OH$ )

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight containers.

## Candesartan Cilexetil



$C_{33}H_{34}N_6O_6$

1*H*-Benzimidazole-7-carboxylic acid, 2-ethoxy-1-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-, 1-[(cyclohexyloxy)carbonyl]oxyethyl ester, (±);  
(±)-1-Hydroxyethyl 2-ethoxy-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester). 610.66  
[145040-37-5].

### DEFINITION

Candesartan Cilexetil contains NLT 98.7% and NMT 101.0% of  $C_{33}H_{34}N_6O_6$ , calculated on anhydrous basis.

### IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K): If the spectra obtained show differences, proceed with the samples prepared as follows. Separately dissolve a quantity of USP Candesartan Cilexetil RS and Candesartan Cilexetil in alcohol. [NOTE—Heating the solution may be necessary for complete dissolution.] Cool the solution in an ice bath, filter the crystals, and dry at 105°.

• **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the procedure for Organic Impurities.



**ASSAY****• PROCEDURE**

**Sample solution:** 8.33 mg/mL of Candesartan Cilexetil in glacial acetic acid

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Potentiometric

**Titrant:** 0.1 N perchloric acid

**Analysis:** Titrate with 0.1 N perchloric acid VS using a blank determination under the same conditions. Each mL of the *Titrant* is equivalent to 61.07 mg of  $C_{33}H_{34}N_6O_6$ .

**Acceptance criteria:** 98.7%–101.0% on the anhydrous basis

**IMPURITIES****Inorganic Impurities**

**• RESIDUE ON IGNITION (281):** NMT 0.1%, determined from a 1-g sample

**Organic Impurities****• PROCEDURE**

**Diluent:** Acetonitrile and water (3:2)

**Solution A:** Acetonitrile, glacial acetic acid, and water (57:1:43)

**Solution B:** Acetonitrile, glacial acetic acid, and water (90:1:10)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
30	0	100

[NOTE—Equilibration for about 10 min may be necessary between injections.]

**System suitability solution:** 0.04 mg/mL of USP Candesartan Cilexetil RS and 0.125 mg/mL of acenaphthene in *Diluent*

**Standard solution:** 4 µg/mL of USP Candesartan Cilexetil RS in *Diluent*

**Sample solution:** 0.4 mg/mL of Candesartan Cilexetil in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 15-cm; 4-µm packing L1

**Flow rate:** 0.8 mL/min

**Injection size:** 10 µL

**System suitability**

[NOTE—The *Mobile phase* used for testing system suitability is 100% *Solution A* in an isocratic mode.]

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 5.0 between candesartan cilexetil and acenaphthene

**Tailing factor:** NMT 1.5 for candesartan cilexetil

**Relative standard deviation:** NMT 3.0% for the candesartan cilexetil peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Candesartan Cilexetil taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of each individual impurity from the *Sample solution*

$r_s$  = peak response of candesartan cilexetil from the *Standard solution*

$C_s$  = concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Candesartan Cilexetil in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** See *Table 1*.

**Total impurities:** NMT 0.6%. [NOTE—Calculate the total impurities from the sum of all impurity peaks greater than or equal to 0.05%.]

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ethyl candesartan <sup>a</sup>	0.4	0.2
Desethyl candesartan cilexetil <sup>b</sup>	0.5	0.3
Candesartan cilexetil	1.0	—
N <sup>2</sup> -Ethyl candesartan cilexetil <sup>c</sup>	2.0	0.2
Any other unknown impurity	—	0.10

<sup>a</sup> Ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylate.

<sup>b</sup> ±1-(Cyclohexyloxy)carbonyloxyethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-oxobenzimidazole-7-carboxylate.

<sup>c</sup> ±1-(Cyclohexyloxy)carbonyloxyethyl 2-ethoxy-1-[[2'-(*N*-ethyl-tetrazol-5-yl)biphenyl-4-yl]methyl]benzimidazole-7-carboxylate.

**SPECIFIC TESTS**

**• WATER DETERMINATION, Method 1 (921):** NMT 0.3%

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.

**• USP REFERENCE STANDARDS (11)**

USP Candesartan Cilexetil RS

1*H*-Benzimidazole-7-carboxylic acid, 2-ethoxy-1-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-, 1-[[[(cyclohexyloxy)carbonyloxy]ethylester, (±); (±)-1-Hydroxyethyl 2-ethoxy-1-[*p*-(o-1*H*-tetrazol-5-ylphenyl)benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester).  
610.66

**Candesartan Cilexetil Tablets****DEFINITION**

Candesartan Cilexetil Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of candesartan cilexetil ( $C_{33}H_{34}N_6O_6$ ).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- B.** The UV absorption spectra of the major peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak from the *Standard solution*, as obtained in the Assay.

**ASSAY****• PROCEDURE**

**Mobile phase:** Acetonitrile, trifluoroacetic acid, and water (550:1:450)

**Diluent:** Acetonitrile and water (70:30)

**Standard solution:** 0.8 mg/mL of USP Candesartan Cilexetil RS in *Diluent*. Sonication may be necessary for complete dissolution. Pass through a suitable filter of 0.45-µm pore size.

**Sample solution:** Nominally 0.8 mg/mL of candesartan cilexetil in *Diluent* prepared as follows. Transfer a number of Tablets (see *Table 1*) to a suitable volumetric flask.



Table 1

Tablet Strength (mg)	Number of Tablets (NLT)
4	10
8	10
16	5
32	5

Add *Diluent* to fill about 70% of the total volume, and sonicate for about 25 min with intermittent shaking. Allow to cool and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detectors

Assay: UV 282 nm

Identification test B: Diode array

Column: 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10  $\mu$ L

Run time: NLT 2.7 times the retention time of candesartan cilexetil

**System suitability**

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of candesartan cilexetil ( $C_{33}H_{34}N_6O_6$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of candesartan cilexetil in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION (711)**

Medium for Tablets labeled to contain 4 mg, 8 mg, and 16 mg: 0.35% Polysorbate 20 in 0.05 M phosphate buffer, pH 6.5; 900 mL

Medium for Tablets labeled to contain 32 mg: 0.70% Polysorbate 20 in 0.05 M phosphate buffer, pH 6.5; 900 mL

Apparatus 2: 50 rpm

Time: 45 min

Mobile phase: Acetonitrile, trifluoroacetic acid, and water (550:1:450)

Standard stock solution: 0.45 mg/mL of USP Candesartan Cilexetil RS in acetonitrile. Sonication may be necessary for complete dissolution.

Standard solution: Prepare solutions in *Medium* from *Standard stock solution* (see *Table 2* for concentrations).

Table 2

Tablet Strength (mg)	Concentration (mg/mL)
4	0.0045
8	0.009
16	0.018
32	0.036

**Sample solution:** Pass a portion of solution under test through a suitable filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 50  $\mu$ L

Run time: NLT 1.8 times the retention time of candesartan cilexetil

**System suitability**

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of candesartan cilexetil ( $C_{33}H_{34}N_6O_6$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$V$  = volume of medium, 900 mL

$L$  = label claim (mg/Tablet)

Tolerances: NLT 80% (Q) of the labeled amount of candesartan cilexetil ( $C_{33}H_{34}N_6O_6$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**IMPURITIES****• ORGANIC IMPURITIES**

**Solution A:** Acetonitrile, trifluoroacetic acid, and water (10: 0.1: 90)

**Solution B:** Acetonitrile, trifluoroacetic acid, and water (90: 0.1: 10)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	65	35
30	5	95
45	5	95
50	65	35
55	65	35

**System suitability stock solution A:** 0.05 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS in acetonitrile

**System suitability stock solution B:** 0.1 mg/mL of USP Candesartan Cilexetil RS in acetonitrile

**System suitability stock solution C:** 0.5 mg/mL of USP Candesartan Cilexetil Related Compound G RS in methanol

**System suitability solution:** 0.0015 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS, 0.001 mg/mL of USP Candesartan Cilexetil RS, 0.005 mg/mL of USP Candesartan Cilexetil Related Compound G RS from *System suitability stock solution A*, *System suitability stock solution B*, and *System suitability stock solution C* in acetonitrile



**Standard solution:** 0.001 mg/mL of USP Candesartan Cilexetil RS in acetonitrile from *System suitability stock solution B*

**Sample solution:** Nominally 1 mg/mL of candesartan cilexetil in acetonitrile prepared as follows. Transfer a suitable quantity of candesartan cilexetil from NLT 20 powdered Tablets into a suitable volumetric flask. Add acetonitrile to fill 60% of the total volume and sonicate for 15 min with intermittent shaking in cold water. Dilute with acetonitrile to volume and pass through a suitable filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  10-cm; 3.5- $\mu$ m packing L1

**Sample cooler temperature:** 10°

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 5.0 between candesartan cilexetil related compound B and candesartan cilexetil, *System suitability solution*

**Tailing factor:** NMT 2.0 for candesartan cilexetil peak, *Standard solution*

**Relative standard deviation:** NMT 10.0% for candesartan cilexetil peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of candesartan cilexetil from the *Standard solution*

$C_S$  = concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of candesartan cilexetil in the *Sample solution* (mg/mL)

$F$  = relative response factor of each impurity (see *Table 4*)

**Acceptance criteria:** See *Table 4*.

**Table 4**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Candesartan cilexetil related compound G <sup>a</sup>	0.17	1.30	1.0
Candesartan cilexetil related compound A <sup>b,c</sup>	0.46	1.16	—
Candesartan cilexetil related compound B <sup>d</sup>	0.77	1.00	1.5
Candesartan cilexetil	1.0	—	—

<sup>a</sup> 1-[[2'-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylic acid.

<sup>b</sup> Ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylate.

<sup>c</sup> Process-related impurity not included in total impurities.

<sup>d</sup> 1-(Cyclohexyloxycarbonyloxy)ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-hydroxybenzimidazole-7-carboxylate.

<sup>e</sup> 1-[[[(Cyclohexyloxy)carbonyl]oxy]ethyl 3-[(2'-2-ethyl-1*H*-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl]methyl]-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.

<sup>f</sup> 1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-[[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl]benzimidazole-7-carboxylate.

**Table 4 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Candesartan cilexetil related compound D <sup>e</sup>	1.15	1.00	0.5
Candesartan cilexetil related compound F <sup>f</sup>	1.47	0.88	1.5
Any unspecified impurity	—	1.00	0.2
Total impurities	—	—	3.0

<sup>a</sup> 1-[[2'-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylic acid.

<sup>b</sup> Ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylate.

<sup>c</sup> Process-related impurity not included in total impurities.

<sup>d</sup> 1-(Cyclohexyloxycarbonyloxy)ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-hydroxybenzimidazole-7-carboxylate.

<sup>e</sup> 1-[[[(Cyclohexyloxy)carbonyl]oxy]ethyl 3-[(2'-2-ethyl-1*H*-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl]methyl]-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.

<sup>f</sup> 1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-[[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl]benzimidazole-7-carboxylate.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

#### • USP REFERENCE STANDARDS (11)

USP Candesartan Cilexetil RS

USP Candesartan Cilexetil Related Compound A RS

Ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylate.

C<sub>26</sub>H<sub>24</sub>N<sub>6</sub>O<sub>3</sub> 468.51

USP Candesartan Cilexetil Related Compound B RS

1-(Cyclohexyloxycarbonyloxy)ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-hydroxybenzimidazole-7-carboxylate.

C<sub>31</sub>H<sub>30</sub>N<sub>6</sub>O<sub>6</sub> 582.61

USP Candesartan Cilexetil Related Compound D RS

1-[[[(Cyclohexyloxycarbonyloxy)carbonyl]oxy]ethyl 3-[[2'-(2-ethyl-2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.

C<sub>33</sub>H<sub>34</sub>N<sub>6</sub>O<sub>6</sub> 610.67

USP Candesartan Cilexetil Related Compound F RS

1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-[[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl]benzimidazole-7-carboxylate.

C<sub>35</sub>H<sub>38</sub>N<sub>6</sub>O<sub>6</sub> 638.71

USP Candesartan Cilexetil Related Compound G RS

1-[[2'-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylic acid.

C<sub>24</sub>H<sub>20</sub>N<sub>6</sub>O<sub>3</sub> 440.45

## Candesartan Cilexetil and Hydrochlorothiazide Tablets

### DEFINITION

Candesartan Cilexetil and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% each of the labeled amount of candesartan cilexetil (C<sub>33</sub>H<sub>34</sub>N<sub>6</sub>O<sub>6</sub>) and hydrochlorothiazide (C<sub>7</sub>H<sub>8</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>).

### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the major peaks of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peaks of the *Standard solution*, as obtained in the *Assay*.



**ASSAY**• **PROCEDURE**

**Solution A:** Acetonitrile, trifluoroacetic acid, and water (10:0.1:90)

**Solution B:** Acetonitrile, trifluoroacetic acid, and water (90:0.1:10)

**Mobile phase:** See Table 1.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	90	10
4.0	90	10
6.0	30	70
15.0	30	70
17.0	90	10
20.0	90	10

**Diluent:** Acetonitrile and water (70:30)

**Standard solution:** Prepare solutions of USP

Candesartan Cilexetil RS and USP Hydrochlorothiazide RS in *Diluent* at concentrations given in Table 2 as follows. Transfer suitable amounts of USP Candesartan Cilexetil RS and USP Hydrochlorothiazide RS to a suitable volumetric flask. Add *Diluent*, about 50% of the total volume, and sonicate to dissolve. Dilute with *Diluent* to volume and pass through a suitable filter of 0.45- $\mu$ m pore size.

**Table 2**

Tablet Strength Candesartan Cilexetil/Hydrochlorothiazide (mg/mg)	Concentration of Candesartan Cilexetil (mg/mL)	Concentration of Hydrochlorothiazide (mg/mL)
16/12.5	0.32	0.25
32/12.5	0.64	0.25
32/25	0.32	0.25

**Sample solution:** Nominally equivalent to the concentration mentioned in Table 2 prepared as follows. Transfer NLT 5 Tablets to a suitable volumetric flask. Add about 60% of the total volume of *Diluent*, and sonicate for about 25 min with intermittent shaking. Cool to room temperature and dilute with *Diluent* to volume. Pass through a suitable filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector**

**Assay:** UV 282 nm

**Identification test B:** Diode array, UV 282 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7. [NOTE—Conditioning of the column with *Solution A* and *Solution B* (80:20) for about 30 min is recommended prior to use.]

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0 for both candesartan cilexetil and hydrochlorothiazide peaks

**Relative standard deviation:** NMT 2.0% for both candesartan cilexetil and hydrochlorothiazide peaks

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount each of candesartan cilexetil ( $C_{33}H_{34}N_6O_6$ ) and hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of candesartan cilexetil or hydrochlorothiazide from the *Sample solution*

$r_S$  = peak response of candesartan cilexetil or hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of USP Candesartan Cilexetil RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of candesartan cilexetil or hydrochlorothiazide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% each of candesartan cilexetil and hydrochlorothiazide

**PERFORMANCE TESTS**• **DISSOLUTION (711)**

**Medium**

For Tablets labeled to contain 16 mg/12.5 mg of candesartan cilexetil/hydrochlorothiazide: 0.35% Polysorbate 20 in 0.05 M phosphate buffer pH 6.5; 900 mL

For Tablets labeled to contain 32 mg/12.5 mg and 32 mg/25 mg of candesartan cilexetil/hydrochlorothiazide: 0.70% Polysorbate 20 in 0.05 M phosphate buffer pH 6.5; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Solution A and Solution B:** Proceed as directed in the Assay.

**Mobile phase:** See Table 3.

**Table 3**

Time (min)	Solution A (%)	Solution B (%)
0	80	20
3.0	80	20
5.0	30	70
10.0	30	70
13.0	80	20
16.0	80	20

**Standard stock solution A:** 0.72 mg/mL of USP

Candesartan Cilexetil RS in acetonitrile prepared as follows. Transfer a quantity of USP Candesartan Cilexetil RS to a suitable volumetric flask. Add acetonitrile, about 50% of volume of the flask, and sonicate. Dilute with acetonitrile to volume.

**Standard stock solution B:** 0.28 mg/mL of USP Hydrochlorothiazide RS in acetonitrile prepared as follows.

Transfer a quantity of USP Hydrochlorothiazide RS to a suitable volumetric flask. Add acetonitrile, about 50% of volume of the flask, and sonicate. Dilute with acetonitrile to volume.

**Standard solution:** Prepare solutions of concentrations per Table 4, from *Standard stock solution A* and *Standard stock solution B* in the appropriate *Medium*. Pass through a suitable filter of 0.45- $\mu$ m pore size.



Table 4

Tablet Strength Candesartan Cilexetil/Hydrochlorothiazide (mg/mg)	Concentration of Candesartan Cilexetil (mg/mL)	Concentration of Hydrochlorothiazide (mg/mL)
16/12.5	0.018	0.014
32/12.5 and 32/25	0.036	0.014

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 264 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7.

[NOTE—Conditioning of the column with *Solution A* and *Solution B* (80:20) for NLT 20 min is recommended prior to use.]

**Flow rate:** 1 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0 for both candesartan cilexetil and hydrochlorothiazide peaks

**Relative standard deviation:** NMT 2.0% for both candesartan cilexetil and hydrochlorothiazide peaks

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of candesartan cilexetil ( $C_{33}H_{34}N_6O_6$ ) or hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times (1/L) \times 100$$

$r_u$  = peak response of candesartan cilexetil or hydrochlorothiazide from the *Sample solution*

$r_s$  = peak response of candesartan cilexetil or hydrochlorothiazide from the *Standard solution*

$C_s$  = concentration of USP Candesartan Cilexetil RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 80% (Q) each of the labeled amounts of candesartan cilexetil ( $C_{33}H_{34}N_6O_6$ ) and hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### Change to read:

- **ORGANIC IMPURITIES**

**Solution A and Solution B:** Proceed as directed in the Assay.

**Mobile phase:** See *Table 5*.

Table 5

Time (min)	Solution A (%)	Solution B (%)
0	95	5
8	95	5
15	60	40
20	60	40
30	40	60
35	30	70
45	20	80

Table 5 (Continued)

Time (min)	Solution A (%)	Solution B (%)
50	0	100
60	0	100
62	95	5
70	95	5

**Diluent A:** Acetonitrile and water (70:30)

**Diluent B:** Acetonitrile and water (50:50)

**Peak identification stock solution A:** 0.05 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS in acetonitrile

**Peak identification stock solution B:** 0.1 mg/mL of USP Candesartan Cilexetil RS in acetonitrile

**Peak identification stock solution C:** 0.5 mg/mL of USP Candesartan Cilexetil Related Compound G RS in methanol

**Peak identification solution:** 0.0015 mg/mL each of USP Candesartan Cilexetil Related Compound A RS, USP Candesartan Cilexetil Related Compound B RS, USP Candesartan Cilexetil Related Compound D RS, and USP Candesartan Cilexetil Related Compound F RS, 0.001 mg/mL of USP Candesartan Cilexetil RS, and 0.005 mg/mL of USP Candesartan Cilexetil Related Compound G RS from *Peak identification stock solution A*, *Peak identification stock solution B*, and *Peak identification stock solution C* in acetonitrile

**System suitability stock solution:** 0.05 mg/mL each of USP Benzothiadiazine Related Compound A RS and USP Hydrochlorothiazide RS, and 0.1 mg/mL of USP Chlorothiazide RS in *Diluent B*

**System suitability solution:** 2.5  $\mu$ g/mL each of USP Benzothiadiazine Related Compound A RS and USP Hydrochlorothiazide RS, and 5  $\mu$ g/mL of USP Chlorothiazide RS in *Diluent A* from *System suitability stock solution*

**Standard stock solution:** 1.6 mg/mL of USP Candesartan Cilexetil RS and 0.6 mg/mL of USP Hydrochlorothiazide RS in *Diluent A* prepared as follows. Transfer a quantity of USP Candesartan Cilexetil RS and USP Hydrochlorothiazide RS to a suitable volumetric flask. Add *Diluent A*, about 60% of the total volume, and sonicate to dissolve. Dilute with *Diluent A* to volume.

**Standard solution:** 0.008 mg/mL of USP Candesartan Cilexetil RS and 0.003 mg/mL of USP Hydrochlorothiazide RS in *Diluent A* from *Standard stock solution*. Pass through a suitable filter of 0.45- $\mu$ m pore size.

**Sample solution:** Nominally 1.5 mg/mL of candesartan cilexetil in acetonitrile prepared as follows. Transfer about 75 mg of candesartan cilexetil, from NLT 20 finely powdered Tablets, to a 50-mL volumetric flask. Add 30 mL of *Diluent A* and sonicate for 20 min with intermittent shaking in cold water. Allow it to reach room temperature, dilute with *Diluent A* to volume, and pass through a suitable filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L7

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*.

[NOTE—The relative retention times for benzothiadiazine related compound A and chlorothiazide in *Table 6* are relative to hydrochlorothiazide.]



**Suitability requirements**

**Resolution:** NLT 1.5 between benzothiadiazine related compound A and chlorothiazide; NLT 1.5 between chlorothiazide and hydrochlorothiazide, *System suitability solution*

**Tailing factor:** NMT 2.0 for both candesartan cilexetil and hydrochlorothiazide peaks, *Standard solution*

**Relative standard deviation:** NMT 10.0% for both candesartan cilexetil and hydrochlorothiazide peaks, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity of candesartan cilexetil and any unspecified impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of candesartan cilexetil from the *Standard solution*

$C_S$  = concentration of USP Candesartan Cilexetil RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of candesartan cilexetil in the *Sample solution* (mg/mL)

$F$  = relative response factor (see Table 6)

Calculate the percentage of benzothiadiazine related compound A and chlorothiazide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of benzothiadiazine related compound A and chlorothiazide from the *Sample solution*

$r_S$  = peak response of hydrochlorothiazide from the *Standard solution*

$C_S$  = concentration of USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydrochlorothiazide in the *Sample solution* (mg/mL)

$F$  = relative response factor (see Table 6)

**Acceptance criteria:** See Table 6.

**Table 6**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Candesartan cilexetil related compound G <sup>a</sup>	0.51	1.11	1.0
Candesartan cilexetil related compound A <sup>b,c</sup> ( <i>ETH 1-Anc-2016</i> )	0.73	1.16	—
Benzothiadiazine related compound A <sup>d</sup>	0.75	1.15	1.0

<sup>a</sup> 1-[[2'-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylic acid.

<sup>b</sup> Ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylate.

<sup>c</sup> Process related impurity not included in *Total impurities*.

<sup>d</sup> 4-Amino-6-chloro-1,3-benzenedisulfonamide.

<sup>e</sup> 6-Chloro-2-*H*-1,2,4-benzothiadiazine-7-sulfonamide-1-1-dioxide.

<sup>f</sup> 1-(Cyclohexyloxycarbonyloxy)ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-hydroxybenzimidazole-7-carboxylate.

<sup>g</sup> 1-[[[(Cyclohexyloxycarbonyloxy)carbonyl]oxy]ethyl 3-[[2'-(2-ethyl-2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.

<sup>h</sup> 1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-[[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl]benzimidazole-7-carboxylate.

**Table 6 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chlorothiazide <sup>a</sup>	0.85	0.48	0.5
Candesartan cilexetil related compound B <sup>f</sup>	0.89	0.90	1.75
Candesartan cilexetil	1.00	—	—
Candesartan cilexetil related compound D <sup>g</sup>	1.06	0.91	0.5
Candesartan cilexetil related compound F <sup>h</sup>	1.24	0.83	1.5
Any unspecified degradation product	—	1.00	0.2
Total impurities	—	—	4.0

<sup>a</sup> 1-[[2'-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylic acid.

<sup>b</sup> Ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylate.

<sup>c</sup> Process related impurity not included in *Total impurities*.

<sup>d</sup> 4-Amino-6-chloro-1,3-benzenedisulfonamide.

<sup>e</sup> 6-Chloro-2-*H*-1,2,4-benzothiadiazine-7-sulfonamide-1-1-dioxide.

<sup>f</sup> 1-(Cyclohexyloxycarbonyloxy)ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-hydroxybenzimidazole-7-carboxylate.

<sup>g</sup> 1-[[[(Cyclohexyloxycarbonyloxy)carbonyl]oxy]ethyl 3-[[2'-(2-ethyl-2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.

<sup>h</sup> 1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-[[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl]benzimidazole-7-carboxylate.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Benzothiadiazine Related Compound A RS

4-Amino-6-chloro-1,3-benzenedisulfonamide.

$C_6H_8ClN_3O_4S_2$  285.73

USP Candesartan Cilexetil RS

USP Candesartan Cilexetil Related Compound A RS

Ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylate.

$C_{26}H_{24}N_6O_3$  468.51

USP Candesartan Cilexetil Related Compound B RS

1-(Cyclohexyloxycarbonyloxy)ethyl 1-[[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-hydroxybenzimidazole-7-carboxylate.

$C_{31}H_{30}N_6O_6$  582.61

USP Candesartan Cilexetil Related Compound D RS

1-[[[(Cyclohexyloxycarbonyloxy)carbonyl]oxy]ethyl 3-[[2'-(2-ethyl-2*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carboxylate.

USP Candesartan Cilexetil Related Compound F RS

1-(Cyclohexyloxycarbonyloxy)ethyl 2-ethoxy-1-[[2'-(2-ethyltetrazol-5-yl)biphenyl-4-yl]methyl]benzimidazole-7-carboxylate.

$C_{35}H_{38}N_6O_6$  638.71

USP Candesartan Cilexetil Related Compound G RS

1-[[2'-(1*H*-Tetrazol-5-yl)biphenyl-4-yl]methyl]-2-ethoxybenzimidazole-7-carboxylic acid.

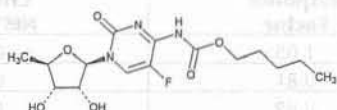
$C_{24}H_{20}N_6O_3$  440.45

USP Chlorothiazide RS

USP Hydrochlorothiazide RS



## Capecitabine



$C_{15}H_{22}FN_3O_6$  359.35

Carbamic acid, [1-(5-deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinyl]-, pentyl ester;  
Pentyl 1-(5-deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinecarbamate [154361-50-9].

### DEFINITION

Capecitabine contains NLT 98.0% and NMT 102.0% of  $C_{15}H_{22}FN_3O_6$ , calculated on the anhydrous and solvent-free basis.

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197K)

Sample: 2 mg of sample in 300 mg of potassium bromide

- B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

Diluent: Methanol, acetonitrile, and water (7:1:12)

Solution A: 0.1% mixture of glacial acetic acid in water

Solution B: Methanol, acetonitrile, and Solution A (7:1:12)

Solution C: Methanol, acetonitrile, and Solution A (16:1:3)

Mobile phase: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
5	100	0
20	49	51
30	49	51
31	100	0
40	100	0

[NOTE—The following solutions may be sonicated if necessary.]

**System suitability solution:** 0.6 μg/mL each of USP Capecitabine RS, USP Capecitabine Related Compound A RS, USP Capecitabine Related Compound B RS, and USP Capecitabine Related Compound C RS in *Diluent*  
**Standard solution:** 0.6 mg/mL of USP Capecitabine RS in *Diluent*

**Sample solution:** 0.6 mg/mL of Capecitabine in *Diluent*

**Chromatographic system** (See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Autosampler temperature: 5°

Flow rate: 1 mL/min

Injection size: 10 μL

**System suitability**

Samples: *System suitability solution* and *Standard solution*

[NOTE—For the purpose of peak identification, the approximate relative retention times are given in *Impurity Table 1*. The relative retention times are measured with respect to capecitabine.]

#### Suitability requirements

**Resolution:** NLT 1.0 between capecitabine related compound A and capecitabine related compound B, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{15}H_{22}FN_3O_6$  in the portion of Capecitabine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Capecitabine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis

### IMPURITIES

#### Inorganic Impurities

- RESIDUE ON IGNITION (281): NMT 0.1%

#### Delete the following:

- HEAVY METALS, Method II (231): NMT 20 ppm (Official 1-Jan-2018)

#### Organic Impurities

##### PROCEDURE

Diluent, Solution B, Solution C, *System suitability solution*, *Standard solution*, *Sample solution*, and *Chromatographic system*: Proceed as directed in the Assay.

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capecitabine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response for capecitabine from the *Standard solution*

$C_S$  = concentration of USP Capecitabine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Capecitabine in the *Sample solution* (mg/mL)

F = relative response factor for an impurity, from *Impurity Table 1*

#### Acceptance criteria

Individual impurities: See *Impurity Table 1*.

Total impurities: NMT 1.5%



Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Capecitabine related compound A	0.18	1.05	0.3
Capecitabine related compound B	0.19	0.81	0.3
2',3'-Di-O-acetyl-5'-deoxy-5-fluorocytidine	0.36	0.89	0.1
5'-Deoxy-5-fluoro-N4-(2-methyl-1-butyloxy-carbonyl)cytidine + 5'-Deoxy-5-fluoro-N4-(3-methyl-1-butyloxy-carbonyl)cytidine	0.95	1.01	0.5
Capecitabine	1.00	1.00	—
[1-[5-Deoxy-3-O-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.06	1.00	0.3
[1-[5-Deoxy-2-O-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.09	1.00	0.2
Capecitabine related compound C	1.11	0.91	0.3
[1-[5-Deoxy-3-O-(5-deoxy-α-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester	1.20	1.00	0.3
2',3'-Di-O-acetyl-5'-deoxy-5-fluoro-N4-(pentyloxycarbonyl)cytidine	1.37	0.85	0.1
Individual unspecified impurity	—	1.00	0.1

**SPECIFIC TESTS**

- **OPTICAL ROTATION**, *Specific Rotation* (781S): +96.0° to +100.0°

Sample solution: 10 mg/mL, on the anhydrous and solvent-free basis, in methanol, at 20°

- **WATER DETERMINATION**, *Method 1c* (921): NMT 0.3%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight containers. Store at controlled room temperature.

- **USP REFERENCE STANDARDS** (11)

USP Capecitabine RS

USP Capecitabine Related Compound A RS

5'-Deoxy-5-fluorocytidine.

C<sub>9</sub>H<sub>12</sub>FN<sub>3</sub>O<sub>4</sub> 245.21

USP Capecitabine Related Compound B RS

5'-Deoxy-5-fluorouridine.

C<sub>9</sub>H<sub>11</sub>FN<sub>2</sub>O<sub>5</sub> 246.19

USP Capecitabine Related Compound C RS

2',3'-O-Carbonyl-5'-deoxy-5-fluoro-N<sup>4</sup>-(pentyloxycarbonyl)cytidine.

C<sub>16</sub>H<sub>20</sub>FN<sub>3</sub>O<sub>7</sub> 385.34

**ASSAY**

- **PROCEDURE**

**Diluent**: Methanol, acetonitrile, and water (7:1:12)

**Solution A**: 0.1% mixture of glacial acetic acid in water

**Solution B**: Methanol, acetonitrile, and *Solution A*

(7:1:12)

**Solution C**: Methanol, acetonitrile, and *Solution A*

(16:1:3)

**Mobile phase**: See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
5	100	0
20	49	51
30	49	51
31	100	0
40	100	0

[NOTE—The following solutions may be sonicated as necessary.]

**System suitability solution**: Includes 0.6 µg/mL of USP Capecitabine RS, 0.6 µg/mL of USP Capecitabine Related Compound A RS, 0.6 µg/mL of USP Capecitabine Related Compound B RS, and 0.6 µg/mL of USP Capecitabine Related Compound C RS in *Diluent*

**Standard solution**: 0.6 mg/mL of USP Capecitabine RS in *Diluent*

**Sample solution**: Equivalent to 0.6 mg/mL of capecitabine, from powdered Tablets (NLT 20), in *Diluent*. [NOTE—Pass through a PVDF membrane filter of 0.45-µm pore size, and use the filtrate.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Capecitabine Tablets****DEFINITION**

Capecitabine Tablets contain NLT 93.0% and NMT 105.0% of the labeled amount of capecitabine (C<sub>15</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>6</sub>).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)

Analytical wave number: 1500–1760 cm<sup>-1</sup>

Sample: Grind 1 Tablet to a fine powder with a mortar and pestle. Mix 1 mg of this sample with 300 mg of potassium bromide.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.



Mode: LC

Detector: UV 250 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 40°

Autosampler temperature: 5°

Flow rate: 1 mL/min

Injection size: 10 μL

#### System suitability

**Samples:** System suitability solution and Standard solution

[NOTE—For the purpose of peak identification, the approximate relative retention times are given in Impurity Table 1. The relative retention times are measured with respect to capecitabine.]

#### Suitability requirements

**Resolution:** NLT 1.0 between capecitabine related compound A and capecitabine related compound B, System suitability solution

**Tailing factor:** NMT 1.5, Standard solution

**Relative standard deviation:** NMT 2.0%, Standard solution

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of C<sub>15</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>6</sub> in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Capecitabine RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of capecitabine in the Sample solution (mg/mL)

**Acceptance criteria:** 93.0%–105.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** Water; 900 mL, degassed

**Apparatus 2:** 50 rpm

**Time:** 30 min

##### Standard solutions

**For Tablets labeled to contain 150 mg:** 17 mg of USP Capecitabine RS in 100 mL of Medium

**For Tablets labeled to contain 500 mg:** 28 mg of USP Capecitabine RS in 50 mL of Medium

**Sample solution:** Pass a portion of the solution under test through a fiberglass filter of 0.45-μm pore size.

**Analysis:** Determine the amount of C<sub>15</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>6</sub> dissolved by selecting a wavelength with appropriate sensitivity between 300 and 330 nm on portions of the Sample solution, suitably diluted with Medium, if necessary, in comparison with the appropriate Standard solution, using a 1-mm quartz cell. Calculate the percentage of C<sub>15</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>6</sub> dissolved in each Tablet:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/L) \times 100$$

$A_U$  = absorbance of the Sample solution

$A_S$  = absorbance of the Standard solution

$C_S$  = concentration of capecitabine in the Standard solution (mg/mL)

$V$  = volume of medium, 900 mL

$L$  = Tablet label claim (mg)

**Tolerances:** NLT 80% (Q) of the labeled amount of C<sub>15</sub>H<sub>22</sub>FN<sub>3</sub>O<sub>6</sub> is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### Organic Impurities

##### • PROCEDURE

**Diluent, Solution A, Solution B, Solution C, Mobile phase, System suitability solution, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

##### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100/F$$

$r_U$  = peak response for each impurity from the Sample solution

$r_S$  = peak response for capecitabine from the Standard solution

$C_S$  = concentration of USP Capecitabine RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of capecitabine in the Sample solution (mg/mL)

$F$  = relative response factor for each impurity, from Impurity Table 1

##### Acceptance criteria

**Individual impurities:** See Impurity Table 1.

**Total degradation products:** NMT 2.0%

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Capecitabine related compound A	0.18	1.05	1.0
Capecitabine related compound B	0.19	0.81	1.0
2',3'-Di-O-acetyl-5'-deoxy-5-fluorocytidine*	0.36	0.89	—
5'-Deoxy-5-fluoro-N4-(2-methyl-1-butyloxy-carbonyl)cytidine + 5'-Deoxy-5-fluoro-N4-(3-methyl-1-butyloxy-carbonyl)cytidine*	0.95	1.01	—
Capecitabine	1.00	1.00	—
[1-[5-Deoxy-3-O-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.06	1.00	—
[1-[5-Deoxy-2-O-(5-deoxy-β-D-ribofuranosyl)-β-D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.09	1.00	—
Capecitabine related compound C	1.11	0.91	0.5

The impurities marked with an "\*" are process impurities and are not included in the total degradation products.



Impurity Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
[1-[5-Deoxy-3-O-(5-deoxy- $\alpha$ -D-ribofuranosyl)- $\beta$ -D-ribofuranosyl]-5-fluoro-2-oxo-1,2-dihydropyrimidin-4-yl]-carbamic acid pentyl ester*	1.20	1.00	—
2',3'-Di-O-acetyl-5'-deoxy-5-fluoro-N4-(pentyloxycarbonyl)cytidine*	1.37	0.85	—
Individual unspecified degradation product	—	1.00	0.1

The impurities marked with an "\*" are process impurities and are not included in the total degradation products.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
  - USP Capecitabine RS
  - USP Capecitabine Related Compound A RS  
5'-Deoxy-5-fluorocytidine.  
C<sub>9</sub>H<sub>12</sub>FN<sub>3</sub>O<sub>4</sub> 245.21
  - USP Capecitabine Related Compound B RS  
5'-Deoxy-5-fluorouridine.  
C<sub>9</sub>H<sub>11</sub>FN<sub>2</sub>O<sub>5</sub> 246.19
  - USP Capecitabine Related Compound C RS  
2',3'-O-Carbonyl-5'-deoxy-5-fluoro-N<sup>4</sup>-(pentyloxycarbonyl)cytidine.  
C<sub>16</sub>H<sub>20</sub>FN<sub>3</sub>O<sub>7</sub> 385.34

### ASSAY

- **PROCEDURE:** Proceed as directed in *Antibiotics—Microbial Assays* (81).  
Acceptance criteria: 700–1050  $\mu$ g/mg

### IMPURITIES

#### Inorganic Impurities

- **RESIDUE ON IGNITION (281):** NMT 3.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

#### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 30 ppm (Official 1-Jan-2018)

### SPECIFIC TESTS

#### CAPREOMYCIN 1 CONTENT

**Solution A:** 0.4 mg/mL of ammonium bisulfate in water. Pass through a filter of 0.5- $\mu$ m or less pore size.  
**Mobile phase:** Methanol and *Solution A* (2:3)

**System suitability solution:** 0.25 mg/mL of USP Capreomycin Sulfate RS in water

**Sample solution:** 0.25 mg/mL of Capreomycin Sulfate in water

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 268 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L10

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times of capreomycin 1A and capreomycin 1B are 0.85 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between capreomycin 1A and capreomycin 1B

**Tailing factor:** NMT 3.5 for the major peaks (capreomycin 1A and capreomycin 1B)

#### Analysis

[NOTE—The chromatographic run time is at least five times the retention time of the capreomycin 1A peak.]

**Sample:** *Sample solution*

Calculate the percentage of capreomycin 1 in the portion of Capreomycin Sulfate taken:

$$\text{Result} = [(r_{1A} + r_{1B})/r_T] \times 100$$

$r_{1A}$  = peak area response of capreomycin 1A

$r_{1B}$  = peak area response of capreomycin 1B

$r_T$  = total response for all peaks

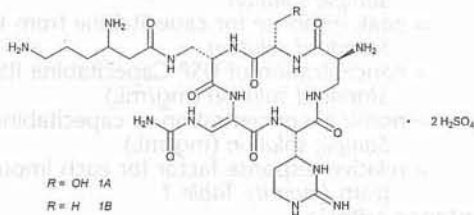
**Acceptance criteria:** NLT 90.0%

- **pH (791):** 4.5–7.5

**Sample solution:** 30 mg/mL

- **LOSS ON DRYING (731):** Dry 100 mg in a vacuum at a pressure not exceeding 5 mm of mercury at 100° for 4 h; it loses NMT 10.0% of its weight.

## Capreomycin Sulfate



Capreomycin sulfate [1405-37-4].

C<sub>25</sub>H<sub>44</sub>N<sub>14</sub>O<sub>8</sub>

668.71

Capreomycin 1A (free base);

3,6-Diamino-N-(((2S,5S,11S,15S,Z)-15-amino-2-(hydroxymethyl)-11-[(R)-iminohexahydropyrimidin-4-yl]-3,6,9,12,16-pentaoxo-8-(ureidomethylene)-1,4,7,10,13-pentaazacyclohexadecan-5-yl)methyl)hexanamide [37290-35-6].

C<sub>25</sub>H<sub>44</sub>N<sub>14</sub>O<sub>7</sub>

652.71

Capreomycin 1B (free base);

3,6-Diamino-N-(((2S,5S,11S,15S,Z)-15-amino-11-[(R)-iminohexahydropyrimidin-4-yl]-2-methyl-3,6,9,12,16-pentaoxo-8-(ureidomethylene)-1,4,7,10,13-pentaazacyclohexadecan-5-yl)methyl)hexanamide [33490-33-4].

### DEFINITION

Capreomycin Sulfate is the disulfate salt of capreomycin, a polypeptide mixture produced by the growth of *Streptomyces capreolus*, suitable for parenteral use. It has a potency equivalent to NLT 700  $\mu$ g/mg and NMT 1050  $\mu$ g/mg of capreomycin.

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Sulfate (191)**
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Capreomycin 1 Content*.



- **BACTERIAL ENDOTOXINS TEST (85):** Where it is intended for use in preparing injectable dosage forms: NMT 0.35 USP Endotoxin Unit/mg of capreomycin
- **OTHER REQUIREMENTS:** Where the label states that Capreomycin Sulfate is sterile, it meets the requirements under *Injections and Implanted Drug Products* (1).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS (11)**  
USP Capreomycin Sulfate RS  
USP Endotoxin RS

### Capreomycin for Injection

#### DEFINITION

Capreomycin for Injection contains an amount of Capreomycin Sulfate equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of capreomycin.

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Sulfate (191)**
- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *System suitability solution*, as obtained in the test for *Capreomycin 1 Content*.

#### ASSAY

- **PROCEDURE:** Proceed as directed in *Antibiotics—Microbial Assays* (81).  
Acceptance criteria: 90.0%–115.0%

#### IMPURITIES

##### Inorganic Impurities

- **RESIDUE ON IGNITION (281):** NMT 3.0%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid

#### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 30 ppm (Official 1-Jan-2018)

#### SPECIFIC TESTS

##### CAPREOMYCIN 1 CONTENT

**Solution A:** 0.4 mg/mL of ammonium bisulfate in water. Pass through a filter of 0.5-μm or less pore size.  
**Mobile phase:** Methanol and *Solution A* (2:3)  
**System suitability solution:** 0.25 mg/mL of USP Capreomycin Sulfate RS in water  
**Sample solution:** 0.25 mg/mL of Capreomycin for Injection in water  
**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)  
**Mode:** LC  
**Detector:** UV 268 nm  
**Column:** 4.6-mm × 25-cm; 5-μm packing L10  
**Column temperature:** 30°  
**Flow rate:** 1.5 mL/min  
**Injection size:** 20 μL  
**System suitability**  
**Sample:** *System suitability solution*  
[NOTE—The relative retention times of capreomycin 1A and capreomycin 1B are 0.85 and 1.0, respectively.]  
**Suitability requirements**  
**Resolution:** NLT 1.5 between capreomycin 1A and capreomycin 1B  
**Tailing factor:** NMT 3.5 for the major peaks (capreomycin 1A and capreomycin 1B)

#### Analysis

[NOTE—The chromatographic run time is at least five times the retention time of the capreomycin 1A peak.]

##### Sample: *Sample solution*

Calculate the percentage of capreomycin 1 in the portion of Capreomycin for Injection taken:

$$\text{Result} = (r_{1A} + r_{1B})/r_T \times 100$$

$r_{1A}$  = peak area response for capreomycin 1A

$r_{1B}$  = peak area response for capreomycin 1B

$r_T$  = total response for all peaks

**Acceptance criteria:** The capreomycin 1 content is NLT 90.0%.

- **PH (791):** 4.5–7.5

**Sample solution:** 30 mg/mL

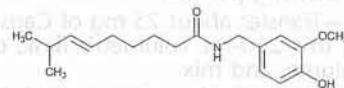
- **LOSS ON DRYING (731):** Dry 100 mg in a vacuum at a pressure not exceeding 5 mm of mercury at 100° for 4 h; it loses NMT 10.0% of its weight.
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.35 USP Endotoxin Unit/mg of capreomycin
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* (1).

#### ADDITIONAL REQUIREMENTS

##### Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).
- **USP REFERENCE STANDARDS (11)**  
USP Capreomycin Sulfate RS  
USP Endotoxin RS

### Capsaicin



$C_{18}H_{27}NO_3$  305.41

6-Nonenamide, (*E*)-*N*-[(4-Hydroxy-3-methoxyphenyl)methyl]-8-methyl.

(*E*)-8-Methyl-*N*-vanillyl-6-nonenamide [404-86-4].

» Capsaicin contains not less than 90.0 percent and not more than 110.0 percent of the labeled percentage of total capsaicinoids. The content of capsaicin ( $C_{18}H_{27}NO_3$ ) is not less than 55 percent, and the sum of the contents of capsaicin and dihydrocapsaicin ( $C_{18}H_{29}NO_3$ ) is not less than 75 percent, and the content of other capsaicinoids is not more than 15 percent, all calculated on the dried basis.

**Caution—Handle Capsaicin with care. Prevent inhalation of particles of it and prevent its contact with any part of the body.**

**Packaging and storage—**Preserve in tight containers, protected from light, and store in a cool place.

**Labeling—**Label it to state the percentage content of total capsaicinoids.



**USP Reference standards (11)—**

USP Capsaicin RS

USP Dihydrocapsaicin RS

**Identification**—Prepare a test solution of Capsaicin in methanol containing 1 mg per mL. Prepare a Standard solution of USP Capsaicin RS in methanol containing 1 mg per mL. Separately apply 10- $\mu$ L portions of the test solution and the Standard solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatograms in a solvent system consisting of a mixture of ether and methanol (19:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow it to air-dry. Spray the plate with a 0.5% solution of 2,6-dibromoquinone-chlorimide in methanol, allow to stand in a chamber containing ammonia fumes, and examine the chromatograms: the blue color and the  $R_f$  value of the principal spot obtained from the test solution correspond to those properties of the principal spot obtained from the Standard solution.

**Melting range** (741): between 57° and 66°, but the range between beginning and end of melting does not exceed 5°.

**Loss on drying** (731): Dry it in vacuum over phosphorus pentoxide at 40° for 5 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 1.0%.

**Content of capsaicin, dihydrocapsaicin, and other capsaicinoids—**

**Mobile phase**—Prepare a mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (600:400). Pass through a filter having a porosity of 0.5  $\mu$ m or finer, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard capsaicin solution**—Quantitatively dissolve an accurately weighed quantity of USP Capsaicin RS in methanol to obtain a solution having a known concentration of about 0.1 mg per mL.

**Standard dihydrocapsaicin solution**—Quantitatively dissolve an accurately weighed quantity of USP Dihydrocapsaicin RS in methanol to obtain a solution having a known concentration of about 0.025 mg per mL.

**Test solution**—Transfer about 25 mg of Capsaicin, accurately weighed, to a 250-mL volumetric flask, dilute with methanol to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 281-nm detector and a 4.6-mm  $\times$  25-cm column that contains 5- $\mu$ m packing L11 and is maintained at a constant temperature of about 30°. Adjust the flow rate to obtain a retention time of about 20 minutes for the main capsaicin peak. Chromatograph the *Standard capsaicin solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard capsaicin solution*, the *Standard dihydrocapsaicin solution*, and the *Test solution* into the chromatograph, record the chromatogram for a period of time that is twice that of the retention time of capsaicin, and measure the areas of the responses for all of the peaks. Calculate the percentage of capsaicin ( $C_{18}H_{27}NO_3$ ) in the portion of Capsaicin taken by the formula:

$$25,000(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Capsaicin RS in the *Standard capsaicin solution*;  $W$  is the weight, in mg, of Capsaicin taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the capsaicin peak responses obtained from the *Test solution* and the *Standard capsaicin solution*, respectively. Not less than 55% is found. Calculate the percentage of

dihydrocapsaicin ( $C_{18}H_{29}NO_3$ ) in the portion of Capsaicin taken by the formula:

$$25,000(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dihydrocapsaicin RS in the *Standard dihydrocapsaicin solution*;  $W$  is the weight, in mg, of Capsaicin taken to prepare the *Test solution*; and  $r_U$  and  $r_S$  are the dihydrocapsaicin peak responses obtained from the *Test solution* and the *Standard dihydrocapsaicin solution*, respectively. The sum of the percentage of capsaicin found and of the percentage of dihydrocapsaicin found is not less than 75%. Using the chromatograms obtained from the *Standard capsaicin solution* and the *Test solution*, calculate the percentage of other capsaicinoids in the portion of Capsaicin taken by the formula:

$$25,000(C/W)(r_T/r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Capsaicin RS in the *Standard capsaicin solution*;  $W$  is the weight, in mg, of Capsaicin taken to prepare the *Test solution*;  $r_T$  is the sum of the peak responses of the capsaicinoids other than capsaicin and dihydrocapsaicin in the chromatogram obtained from the *Test solution*; and  $r_S$  is the capsaicin peak response obtained from the *Standard capsaicin solution*. Not more than 15% of other capsaicinoids is found.

**Capsicum****DEFINITION**

Capsicum is the dried ripe fruit of various *Capsicum* species (Fam. Solanaceae). It contains NLT 0.3% of total capsaicinoids, calculated as the sum of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanilylvanillinamide, and homocapsaicin; and the nonivamide content is NMT 5% of the total capsaicinoids; all calculated on the dried basis.

**IDENTIFICATION****• A. THIN-LAYER CHROMATOGRAPHY**

**Standard solution A:** 0.4 mg/mL of USP Capsaicin RS in methanol

**Standard solution B:** 0.4 mg/mL of USP Dihydrocapsaicin RS in methanol

**Sample solution:** Shake for 5 min about 0.5 g of Capsicum, finely powdered, in 5 mL of hexanes, and centrifuge. Use the supernatant.

**Chromatographic system**

**Adsorbent:** Chromatographic reversed-phase octadecyl silyl silica gel with an average particle size of 5  $\mu$ m (HPTLC plates)

**Application volume:** 2  $\mu$ L, as 8-mm bands

**Developing solvent system:** A mixture of methanol and water (8:2)

**Derivatization reagent A:** 0.25 mg/mL of dichloroquinonechlorimide in ethyl acetate

**Derivatization reagent B:** Ammonium hydroxide solution

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable high-performance thin-layer chromatographic plate. Use a saturated chamber, and condition the plate to a relative humidity of about 33% using a suitable device. Develop the chromatograms over a distance of 6 cm. Remove the plate from the chamber, dry, derivatize with *Derivatization reagent A*, and expose to vapors of *Derivatization reagent B* until blue bands develop. Examine under white light.



**System suitability:** *Standard solution A* shows a blue band at about one-third of the chromatogram, and *Standard solution B* shows a blue band at an  $R_f$  right below that from *Standard solution A*.

**Acceptance criteria:** The *Sample solution* exhibits a blue band at about one-third of the chromatogram, similar in position and color to the capsaicin band in the chromatogram of *Standard solution A*, and exhibits a blue band at an  $R_f$  right below that of capsaicin, similar in position and color to the dihydrocapsaicin band in the chromatogram of *Standard solution B*. Other bands may be observed in the *Sample solution* chromatogram.

#### • B. HPLC

**Analysis:** Proceed as directed in the test for *Content of Total Capsaicinoids*.

**Acceptance criteria:** The *Sample solution* chromatogram exhibits the main capsaicinoid peak at the retention time corresponding to capsaicin in the chromatogram of *Standard solution A* and a peak of lower intensity corresponding to dihydrocapsaicin in the chromatogram of *Standard solution B*. The *Sample solution* chromatogram shows additional minor peaks corresponding to nordihydrocapsaicin, nonivamide, decanylevanillinamide, and homocapsaicin.

#### COMPOSITION

##### • CONTENT OF TOTAL CAPSAICINOIDS

**Mobile phase:** A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (2:3)

**Standard solution A:** 0.02 mg/mL of USP Capsaicin RS in methanol

**Standard solution B:** 0.01 mg/mL of USP Dihydrocapsaicin RS in methanol

**Sample solution:** To a glass centrifuge tube transfer about 0.5 g of Capsicum, powdered and accurately weighed, add 30 mL of methanol, shake for 15 min, and centrifuge. Transfer the supernatant to a 50-mL volumetric flask. To the residue add 10 mL of methanol, shake for 5 min, and centrifuge. Transfer the supernatant to the volumetric flask. Repeat the extraction one more time with 10 mL of methanol. Complete with methanol to volume, and mix. Before injection, pass through a membrane filter of 0.45- $\mu$ m or finer pore size, discarding the first few mL of the filtrate.

##### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 281 nm

**Column:** 4.6-mm  $\times$  25-cm; end-capped, 5- $\mu$ m, 150 Å, packing L11

**Column temperature:** 30°

**Flow rate:** 1.0 mL/min

**Injection volume:** 20  $\mu$ L

##### System suitability

**Samples:** *Standard solution A* and *Sample solution*

##### Suitability requirements

**Resolution:** NLT 1.5 between the capsaicin peak and the nonivamide peak occurring at a retention time of 0.95 relative to 1.0 for capsaicin, *Sample solution*

**Relative standard deviation:** NMT 2.0% for the capsaicin peak in repeated injections, *Standard solution A*

##### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Identify the capsaicin and dihydrocapsaicin peaks in the *Sample solution* chromatogram by comparison with the chromatograms of *Standard solution A* and *Standard solution B*, respectively. Identify the peaks corresponding to nordihydrocapsaicin, nonivamide, decanylevanillinamide, and homocapsaicin using the approximate relative retention times provided in *Table 1*.

**Table 1**

Analyte	Approximate Relative Retention Time
Nordihydrocapsaicin	0.89
Nonivamide	0.95
Capsaicin	1.00
Decanylevanillinamide	1.34
Homocapsaicin	1.40

Calculate the percentage of capsaicin in the portion of Capsicum taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

$r_U$  = peak area of capsaicin from the *Sample solution*

$r_S$  = peak area of capsaicin from *Standard solution A*

$C_S$  = concentration of capsaicin in *Standard solution A* (mg/mL)

$V$  = volume of the *Sample solution* (mL)

$W$  = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the percentage of dihydrocapsaicin in the portion of Capsicum taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

$r_U$  = peak area of dihydrocapsaicin from the *Sample solution*

$r_S$  = peak area of dihydrocapsaicin from *Standard solution B*

$C_S$  = concentration of dihydrocapsaicin in *Standard solution B* (mg/mL)

$V$  = volume of the *Sample solution* (mL)

$W$  = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the percentage of nonivamide, expressed as capsaicin, in the portion of Capsicum taken:

$$\text{Result} = (r_U/r_S) \times C_S \times (V/W) \times 100$$

$r_U$  = peak area of nonivamide from the *Sample solution*

$r_S$  = peak area of capsaicin from *Standard solution A*

$C_S$  = concentration of capsaicin in *Standard solution A* (mg/mL)

$V$  = volume of the *Sample solution* (mL)

$W$  = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the sum of the percentages of nordihydrocapsaicin, decanylevanillinamide, and homocapsaicin, expressed as capsaicin, in the portion of Capsicum taken:

$$\text{Result} = (\Sigma r_U/r_S) \times C_S \times (V/W) \times 100$$

$\Sigma r_U$  = sum of peak areas of nordihydrocapsaicin, decanylevanillinamide, and homocapsaicin from the *Sample solution*

$r_S$  = peak area of capsaicin from *Standard solution A*

$C_S$  = concentration of capsaicin in *Standard solution A* (mg/mL)

$V$  = volume of the *Sample solution* (mL)

$W$  = weight of Capsicum taken to prepare the *Sample solution* (mg)

Calculate the content of total capsaicinoids as the sum of the percentages of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanylevanillinamide, and homocapsaicin.



Acceptance criteria: NLT 0.3% on the dried basis

## CONTAMINANTS

### • ELEMENTAL IMPURITIES—PROCEDURES (233)

Acceptance criteria

Arsenic: NMT 0.5 µg/g

Cadmium: NMT 1.0 µg/g

Lead: NMT 5.0 µg/g

Mercury: NMT 0.1 µg/g

### • ARTICLES OF BOTANICAL ORIGIN, General Method for Pesticide Residues Analysis (561): Meets the requirements

### • ARTICLES OF BOTANICAL ORIGIN, Test for Aflatoxins (561): Meets the requirements

## SPECIFIC TESTS

### • LIMIT OF NONIVAMIDE

**Analysis:** Use the chromatograms and calculations obtained in the test for *Content of Total Capsaicinoids*.

Calculate the content of nonivamide as a percentage of total capsaicinoids:

$$\text{Result} = (\text{PN}/\text{PTC}) \times 100$$

PN = percentage of nonivamide as calculated in the *Content of Total Capsaicinoids*

PTC = percentage of total capsaicinoids as calculated in the *Content of Total Capsaicinoids*

Acceptance criteria: NMT 5% on the dried basis

### • BOTANIC CHARACTERISTICS

**Macroscopic:** Mature Capsicum fruit vary in length from a few cm to more than 10 cm; fruit diameter ranges from a few mm to a few cm; shape varies from elongate, ellipse, almost round, triangular, campanulate, tomato-like, or square (blocky); number of locules two, three, or four; the dissepiments being united at the base to a conical, central placenta; calyx margin is either entire, dentate, or intermediate dentate, and sometimes attached to a long, straight pedicel; the presence of calyx annular constriction, at junction of calyx and pedicel indicates *Capsicum chinense*; the seeds are light brown to weak yellowish-orange, suborbicular or irregular, flattened, from 2–4 mm in diameter, with a thickened edge and a prominent, pointed micropyle.

**Microscopic:** Powdered Capsicum shows fragments of epicarp cells, polygonal, triangular, rectangular, or irregular cells; with or without beaded walls; numerous fragments of thin-walled parenchyma cells of the pericarp containing oil globules and chromoplasts of the various colors similar to the color of the fruits; fragments of sclerenchymatous cells of the endocarp with slightly wavy, lignified walls and broad lumina, striations and pit canal distinct, cells appear bright white under a polarizing microscope; fragments of epidermal cells of testa, extremely thick wall, deeply sinuate, striations and pit canal distinct, cells appear bright yellowish-white under a polarizing microscope; fragments of parenchyma cells of the endosperm containing fixed oil and aleurone grains; and occasional fibrovascular elements and calyx tissues.

### • ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter (561): NMT 1%, other than stems and calyces, the proportion of which does not exceed 3%

### • LOSS ON DRYING (731)

**Sample:** 1.0 g of finely powdered Capsicum

**Analysis:** Dry at 105° for 2 h.

Acceptance criteria: NMT 11%

### • ARTICLES OF BOTANICAL ORIGIN, Total Ash (561): NMT 10.0%

### • ARTICLES OF BOTANICAL ORIGIN, Acid-Insoluble Ash (561): NMT 1.25%

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in well-closed containers. A few drops of chloroform may be added from time to time to prevent attack by insects.

### • LABELING: Label each container to indicate which variety of Capsicum is contained therein.

### • USP REFERENCE STANDARDS (11)

USP Capsaicin RS

USP Dihydrocapsaicin RS

## Capsicum Oleoresin

### DEFINITION

Capsicum Oleoresin is an alcoholic extract of the dried ripe fruits of Capsicum. It contains NLT 6.5% of total capsaicinoids, calculated as the sum of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanilylvanillinamide, and homocapsaicin, all calculated on the anhydrous basis. The nonivamide content is NMT 5% of the total capsaicinoids, calculated on the anhydrous basis.

**[CAUTION—**Capsicum Oleoresin is a powerful irritant, and even in minute quantities produces an intense burning sensation when it comes in contact with the eyes and tender parts of the skin. Care should be taken to protect the eyes and to prevent contact of the skin with Capsicum Oleoresin.]

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHY

**Standard solution A:** 0.4 mg/mL of USP Capsaicin RS in methanol

**Standard solution B:** 0.4 mg/mL of USP Dihydrocapsaicin RS in methanol

**Sample solution:** 10 mg/mL of Capsicum Oleoresin in hexanes

#### Chromatographic system

**Adsorbent:** Chromatographic reverse phase octadecyl silyl silica gel with an average particle size of 5 µm (HPTLC plates)

**Application volume:** 2 µL, as 8-mm bands

**Developing solvent system:** A mixture of methanol and water (8:2)

**Derivatization reagent A:** 0.25 mg/mL of dichloroquinonechlorimide in ethyl acetate

**Derivatization reagent B:** Ammonium hydroxide solution

#### Analysis

**Samples:** Standard solution A, Standard solution B, and Sample solution

Apply the Samples as bands to a suitable high-performance thin-layer chromatographic plate. Use a saturated chamber, and condition the plate to a relative humidity of about 33% using a suitable device. Develop the chromatograms over a distance of 6 cm. Remove the plate from the chamber, dry, derivatize with Derivatization reagent A, dry, expose to vapors of Derivatization reagent B until blue bands develop, and examine under white light.

**System suitability:** Standard solution A shows a blue band at about one-third of the chromatogram, and Standard solution B shows a blue band at an  $R_f$  right below that from Standard solution A.

**Acceptance criteria:** The Sample solution exhibits a blue band at about one-third of the chromatogram, similar in position and color to the capsaicin band in the chromatogram of Standard solution A, and exhibits a blue band at an  $R_f$  right below that of capsaicin, similar in position and color to the dihydrocapsaicin band in the chromatogram of Standard solution B. Other bands may be observed in the Sample solution chromatogram.

#### • B. HPLC

**Analysis:** Proceed as directed in the test for *Content of Total Capsaicinoids*.

**Acceptance criteria:** The Sample solution chromatogram exhibits the main capsaicinoid peak at the retention time corresponding to capsaicin in the chromatogram.



gram of *Standard solution A* and a peak of lower intensity corresponding to dihydrocapsaicin in the chromatogram of *Standard solution B*. The *Sample solution* chromatogram shows additional minor peaks corresponding to nordihydrocapsaicin, nonivamide, decanylevanillinamide, and homocapsaicin.

## ASSAY

### • CONTENT OF TOTAL CAPSAICINOIDS

**Mobile phase:** A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (2:3)

**Standard solution A:** 0.2 mg/mL of USP Capsaicin RS in methanol

**Standard solution B:** 0.1 mg/mL of USP Dihydrocapsaicin RS in methanol

**Sample solution:** 5 mg/mL of Capsicum Oleoresin in methanol. Pass a portion of this solution through a filter of 0.2-μm pore size, and use the filtrate as the *Sample solution*.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 281 nm

**Column:** 4.6-mm × 25-cm; end-capped, 5-μm, 150 Å, packing L11

**Column temperature:** 30°

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 μL

### System suitability

**Samples:** *Standard solution A* and *Sample solution*

### Suitability requirements

**Resolution:** NLT 1.5 between the capsaicin peak and the nonivamide peak that occurs at a retention time of 0.95 relative to 1.0 for capsaicin, *Sample solution*

**Relative standard deviation:** NMT 2.0% for the capsaicin peak, *Standard solution A*

### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Identify the capsaicin and dihydrocapsaicin peaks in the *Sample solution* chromatogram by comparison with the chromatograms of *Standard solution A* and *Standard solution B*, respectively. Identify the peaks corresponding to nordihydrocapsaicin, nonivamide, decanylevanillinamide, and homocapsaicin using the approximate relative retention times provided in *Table 1*.

Table 1

Analyte	Approximate Relative Retention Time
Nordihydrocapsaicin	0.89
Nonivamide	0.95
Capsaicin	1.00
Decanylevanillinamide	1.34
Homocapsaicin	1.40

Calculate the percentage of capsaicin in the portion of Capsicum Oleoresin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of capsaicin from the *Sample solution*

$r_S$  = peak area of capsaicin from *Standard solution A*

$C_S$  = concentration of capsaicin in *Standard solution A* (mg/mL)

$C_U$  = concentration of Capsicum Oleoresin in the *Sample solution* (mg/mL)

Calculate the percentage of dihydrocapsaicin in the portion of Capsicum Oleoresin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of dihydrocapsaicin from the *Sample solution*

$r_S$  = peak area of dihydrocapsaicin from *Standard solution B*

$C_S$  = concentration of dihydrocapsaicin in *Standard solution B* (mg/mL)

$C_U$  = concentration of Capsicum Oleoresin in the *Sample solution* (mg/mL)

Calculate the percentage of nonivamide, expressed as capsaicin, in the portion of Capsicum Oleoresin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of nonivamide from the *Sample solution*

$r_S$  = peak area of capsaicin from *Standard solution A*

$C_S$  = concentration of capsaicin in *Standard solution A* (mg/mL)

$C_U$  = concentration of Capsicum Oleoresin in the *Sample solution* (mg/mL)

Calculate the sum of the percentages of nordihydrocapsaicin, decanylevanillinamide, and homocapsaicin, expressed as capsaicin, in the portion of Capsicum Oleoresin taken:

$$\text{Result} = (\Sigma r_U/r_S) \times (C_S/C_U) \times 100$$

$\Sigma r_U$  = sum of the peak areas of nordihydrocapsaicin, decanylevanillinamide, and homocapsaicin from the *Sample solution*

$r_S$  = peak area of capsaicin from *Standard solution A*

$C_S$  = concentration of capsaicin in *Standard solution A* (mg/mL)

$C_U$  = concentration of Capsicum Oleoresin in the *Sample solution* (mg/mL)

Calculate the content of total capsaicinoids as the sum of the percentages of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanylevanillinamide, and homocapsaicin.

**Acceptance criteria:** NLT 6.5% on the anhydrous basis

## CONTAMINANTS

### • ELEMENTAL IMPURITIES—PROCEDURES (233)

#### Acceptance criteria

Arsenic: NMT 0.5 μg/g

Cadmium: NMT 1.0 μg/g

Lead: NMT 5.0 μg/g

Mercury: NMT 0.1 μg/g

### • ARTICLES OF BOTANICAL ORIGIN, General Method for Pesticide Residues Analysis (561): Meets the requirements

### • ARTICLES OF BOTANICAL ORIGIN, Test for Aflatoxins (561): Meets the requirements

## SPECIFIC TESTS

### • LIMIT OF NONIVAMIDE

**Analysis:** Use the chromatograms and calculations obtained in the test for *Content of Total Capsaicinoids*.

Calculate the content of nonivamide as percentage of total capsaicinoids:

$$\text{Result} = (PN/PTC) \times 100$$

$PN$  = percentage of nonivamide as calculated in the test for *Content of Total Capsaicinoids*

$PTC$  = percentage of total capsaicinoids as calculated in the test for *Content of Total Capsaicinoids*



- Acceptance criteria: NMT 5% on the anhydrous basis
- **WATER DETERMINATION, Method Ia (921)**  
Sample: 5.0 g of Capsicum Oleoresin  
Acceptance criteria: NMT 8%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that if separation occurs, it should be warmed and mixed before use.
- **USP REFERENCE STANDARDS (11)**  
USP Capsaicin RS  
USP Dihydrocapsaicin RS

**Capsicum Tincture****DEFINITION**

Capsicum Tincture is prepared as follows.

Capsicum	100 g
A mixture of Alcohol and Water (70:30) to (85:15), a sufficient quantity to make	1000 mL

Prepare the Tincture as directed for *Botanical Extracts* (565), *Tinctures, Maceration Process*. It contains NLT 0.02% (w/v) of total capsaicinoids, calculated as the sum of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanilyvanillinamide, and homocapsaicin; and the nonivamide content is NMT 5% of the total capsaicinoids.

**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHY**

**Standard solution A:** 0.2 mg/mL of USP Capsaicin RS in methanol

**Standard solution B:** 0.2 mg/mL of USP Dihydrocapsaicin RS in methanol

**Sample solution:** Shake 10 mL of Tincture with 10 mL of hexanes, allow to separate, and use the lower layer.

**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** Chromatographic reverse phase octadecyl silyl silica gel with an average particle size of 5  $\mu$ m (HPTLC plates)

**Application volume:** 2  $\mu$ L, as 8-mm bands

**Relative humidity:** Condition the plate to a relative humidity of about 33% using a suitable device.

**Developing solvent system:** A mixture of methanol and water (8:2)

**Derivatization reagent A:** 5 mg/mL of dichloroquinonechlorimide in methanol

**Derivatization reagent B:** Ammonium hydroxide solution

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply the *Samples* as bands to a suitable high-performance thin-layer chromatographic plate. Use a saturated chamber, and develop the chromatograms over a distance of 6 cm. Remove the plate from the chamber, dry, derivatize with *Derivatization reagent A*, dry, expose to the vapors of *Derivatization reagent B* until blue bands develop, and then examine under white light.

**System suitability:** *Standard solution A* shows a blue band at about one-third of the chromatogram, and *Standard solution B* shows a blue band at an  $R_f$  just below that from *Standard solution A*. Separation between capsaicin and dihydrocapsaicin should be achieved with the reference standards; the bands appear at the intended location of the plate and they are visible as blue bands.

**Acceptance criteria:** The *Sample solution* chromatogram exhibits a blue band at about one-third of the chromatogram, corresponding in color and  $R_f$  to the capsaicin band in the chromatogram of *Standard solution A*, and exhibits a blue band at an  $R_f$  just below that of capsaicin, similar in position and color to the dihydrocapsaicin band in the chromatogram of *Standard solution B*. Other bands may be observed in the *Sample solution* chromatogram.

• **B. HPLC**

**Analysis:** Proceed as directed in the test for *Content of Total Capsaicinoids*.

**Acceptance criteria:** The *Sample solution* chromatogram exhibits the main capsaicinoid peak at the retention time corresponding to capsaicin in the chromatogram of *Standard solution A* and a peak of lower intensity corresponding to dihydrocapsaicin in the chromatogram of *Standard solution B*. The *Sample solution* chromatogram shows additional minor peaks corresponding to nordihydrocapsaicin, nonivamide, decanilyvanillinamide, and homocapsaicin.

**STRENGTH**• **CONTENT OF TOTAL CAPSAICINOIDS**

**Mobile phase:** A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (2:3)

**Standard solution A:** 0.1 mg/mL of USP Capsaicin RS in methanol

**Standard solution B:** 0.05 mg/mL of USP Dihydrocapsaicin RS in methanol

**Sample solution:** Dilute an accurately measured volume of Tincture in methanol (1:1), and mix. Before injection, pass through a membrane filter with a 0.45- $\mu$ m or finer pore size, discarding the first few mL of the filtrate. [NOTE—The sample can be weighed and converted to volume using the density of the Tincture.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 281 nm

**Column:** 4.6-mm  $\times$  25-cm; end-capped, 5- $\mu$ m, 150 Å, packing L11

**Flow rate:** 1.0 mL/min

**Column temperature:** 30°

**Injection volume:** 20  $\mu$ L

**System suitability**

**Samples:** *Standard solution A* and *Sample solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between the capsaicin peak and the nonivamide peak at a retention time of 0.95, relative to 1.0 for capsaicin, *Sample solution*

**Relative standard deviation:** NMT 2.0% for the capsaicin peak in repeated injections, *Standard solution A*

**Analysis**

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Identify the capsaicin and dihydrocapsaicin peaks in the *Sample solution* chromatogram by comparison with the chromatograms of *Standard solution A* and *Standard solution B*, respectively. Identify the peaks corresponding to nordihydrocapsaicin, nonivamide, decanilyvanillinamide, and homocapsaicin using the approximate relative retention times provided in *Table 1*.

**Table 1**

Analyte	Approximate Relative Retention Time
Nordihydrocapsaicin	0.89
Nonivamide	0.95
Capsaicin	1.00
Decanilyvanillinamide	1.34
Homocapsaicin	1.40



Calculate the percentage of capsaicin in the portion of Tincture taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak area of capsaicin from the *Sample solution*  
 $r_S$  = peak area of capsaicin from *Standard solution A*  
 $C_S$  = concentration of capsaicin in *Standard solution A* (g/mL)  
 $C_U$  = concentration of Tincture in the *Sample solution* (mL/mL)

Calculate the percentage of dihydrocapsaicin in the portion of Tincture taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak area of dihydrocapsaicin from the *Sample solution*  
 $r_S$  = peak area of dihydrocapsaicin from *Standard solution B*  
 $C_S$  = concentration of dihydrocapsaicin in *Standard solution B* (g/mL)  
 $C_U$  = concentration of Tincture in the *Sample solution* (mL/mL)

Calculate the percentage of nonivamide, expressed as capsaicin, in the portion of Tincture taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak area of nonivamide from the *Sample solution*  
 $r_S$  = peak area of capsaicin from *Standard solution A*  
 $C_S$  = concentration of capsaicin in *Standard solution A* (g/mL)  
 $C_U$  = concentration of Tincture in the *Sample solution* (mL/mL)

Calculate the sum of the percentages of nordihydrocapsaicin, decanilyvanillinamide and homocapsaicin, expressed as capsaicin, in the portion of Tincture taken:

$$\text{Result} = (\Sigma r_U/r_S) \times (C_S/C_U) \times 100$$

- $\Sigma r_U$  = sum of the peak areas of nordihydrocapsaicin, decanilyvanillinamide, and homocapsaicin from the *Sample solution*  
 $r_S$  = peak area of capsaicin from *Standard solution A*  
 $C_S$  = concentration of capsaicin in *Standard solution A* (g/mL)  
 $C_U$  = concentration of Tincture in the *Sample solution* (mL/mL)

Calculate the content of total capsaicinoids as the sum of the percentages of capsaicin, dihydrocapsaicin, nordihydrocapsaicin, nonivamide, decanilyvanillinamide, and homocapsaicin.

Acceptance criteria: NLT 0.02%

#### OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method I (611):** NLT 90.0% and NMT 110.0% of the labeled amount of  $C_2H_5OH$

#### CONTAMINANTS

- **ARTICLES OF BOTANICAL ORIGIN, General Method for Pesticide Residues Analysis (561):** Meets the requirements

#### SPECIFIC TESTS

##### • LIMIT OF NONIVAMIDE

**Analysis:** Use the chromatograms and calculations obtained in the test for *Content of Total Capsaicinoids*.

Calculate the content of nonivamide as a percentage of total capsaicinoids:

$$\text{Result} = (PN/PTC) \times 100$$

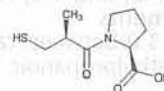
- $PN$  = percentage of nonivamide as calculated in the *Content of Total Capsaicinoids*  
 $PTC$  = percentage of total capsaicinoids as calculated in the *Content of Total Capsaicinoids*

Acceptance criteria: NMT 5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at room temperature.
- **LABELING:** The label states the official name of the article, the Latin binomial, and the part of the plant from which the article was prepared. Label it to indicate the content of capsaicinoids, the solvent mixture used for extraction, and the ratio of the starting crude plant material to Tincture.
- **USP REFERENCE STANDARDS (11)**  
 USP Capsaicin RS  
 USP Dihydrocapsaicin RS

## Captopril



$C_9H_{15}NO_3S$  217.29  
 L-Proline, 1-[(2S)-3-mercapto-2-methyl-1-oxopropyl]-;  
 1-[(2S)-3-Mercapto-2-methylpropionyl]-L-proline  
 [62571-86-2].

#### DEFINITION

Captopril contains NLT 97.5% and NMT 102.0% of captopril ( $C_9H_{15}NO_3S$ ), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**

#### ASSAY

##### • PROCEDURE

**Sample solution:** Dissolve about 300 mg of Captopril in 100 mL of water in a suitable glass-stoppered flask. Add 10 mL of 3.6 N sulfuric acid, 1 g of potassium iodide, and 2 mL of starch TS.

##### Titrimetric system

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** Dissolve 3.567 g of potassium iodate, previously dried at 110° to constant weight, in water to make 1.0 L.

**Endpoint detection:** Visual

**Analysis:** Titrate with *Titrant* to a faint blue endpoint that persists for NLT 30 s. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 21.73 mg of captopril ( $C_9H_{15}NO_3S$ ).

Acceptance criteria: 97.5%–102.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.2%

#### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 30 ppm • (Official 1-

Jan-2018)



### • ORGANIC IMPURITIES

Use low-actinic glassware to prepare the *Standard solution* and *Sample solution*.

**Mobile phase:** 9-in-100 solution of tetrahydrofuran in methanol and 1-in-2000 solution of phosphoric acid (33:67)

**System suitability stock solution:** 0.1 mg/mL each of USP Captopril RS, USP Captopril Disulfide RS, and 3-acetylthio-2-methylpropanoic acid in methanol

**System suitability solution:** 10 µg/mL each of USP Captopril RS, USP Captopril Disulfide RS, and 3-acetylthio-2-methylpropanoic acid in methanol from *System suitability stock solution*

**Standard solution:** 10 µg/mL of USP Captopril Disulfide RS in methanol

**Sample solution:** 2 mg/mL of Captopril in methanol. Use the solution promptly.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for captopril, 3-acetylthio-2-methylpropanoic acid, and captopril disulfide are 0.32, 0.42, and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 3.0 between captopril and 3-acetylthio-2-methylpropanoic acid

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Compare the peak responses from the *Sample solution*, excluding those of the solvent, captopril, and captopril disulfide, with the main peak response from the *Standard solution*.

Calculate the percentage of captopril disulfide in the portion of Captopril taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of captopril disulfide from the *Sample solution*

$r_S$  = peak response of captopril disulfide from the *Standard solution*

$C_S$  = concentration of USP Captopril Disulfide RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Captopril in the *Sample solution* (µg/mL)

**Acceptance criteria:** NMT 1.0% of captopril disulfide. The peak response of each impurity does not exceed 40% of the main peak response from the *Standard solution* (0.2%), and the sum of the impurity peak responses does not exceed the main peak response from the *Standard solution* (0.5%).

### SPECIFIC TESTS

#### • OPTICAL ROTATION, *Specific Rotation* (781S)

**Sample solution:** 10 mg/mL in dehydrated alcohol

**Acceptance criteria:** −125° to −134°

#### • LOSS ON DRYING (731)

**Analysis:** Dry a sample under vacuum at 60° for 3 h.

**Acceptance criteria:** NMT 1.0%

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE:

Preserve in tight containers.

#### • USP REFERENCE STANDARDS (11)

USP Captopril RS

USP Captopril Disulfide RS

L-Proline, 1,1'-[dithiobis(2-methyl-1-oxo-3,1-propanediyl)]bis-[S-(R\*,R\*)]-.

C<sub>18</sub>H<sub>28</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub> 432.55

## Captopril Compounded Oral Solution

### DEFINITION

Captopril Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of captopril (C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S).

Prepare Captopril Compounded Oral Solution 0.75 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Captopril powder	75 mg
Vehicle for Oral Solution (regular or sugar-free), NF, a sufficient quantity to make	100 mL

Add *Captopril powder* and 10 mL of *Vehicle* to a mortar, and mix. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Methanol and water (55:45) containing 0.5 mL/L of phosphoric acid. Filter, and degas.

**Standard solution:** 7.5 µg/mL of USP Captopril RS

**Sample solution:** Agitate the container of Oral Solution for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at −70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for captopril is about 5.0 min.]

### Suitability requirements

**Relative standard deviation:** NMT 0.9% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of captopril (C<sub>9</sub>H<sub>15</sub>NO<sub>3</sub>S) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Captopril RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of captopril in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

### SPECIFIC TESTS

#### • pH (791):

3.8–4.3

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator.

• **BEYOND-USE DATE:** NMT 7 days after the date on which it was compounded when stored in a refrigerator



- **LABELING:** Label it to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS (11)**  
USP Captopril RS

## Captopril Compounded Oral Suspension

### DEFINITION

Captopril Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of captopril ( $C_9H_{15}NO_3S$ ).

Prepare Captopril Compounded Oral Suspension 0.75 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Captopril	75 mg
Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL

Place the required number of tablets in a suitable mortar and comminute to a fine powder, or use *Captopril* powder. Add 10 mL of *Vehicle*, and mix to form a uniform paste. Add the *Vehicle* in small portions, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add sufficient *Vehicle* to bring to final volume, and mix well.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Methanol and water (55:45) containing 0.5 mL/L of phosphoric acid. Filter, and degas.

**Standard solution:** 7.5 µg/mL of USP Captopril RS

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at  $-70^\circ$  until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 1.0 mL of the sample into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for captopril is about 5.0 min.]

#### Suitability requirements

**Relative standard deviation:** NMT 0.9% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of captopril ( $C_9H_{15}NO_3S$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Captopril RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of captopril in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

### SPECIFIC TESTS

- **PH (791):** 3.8–4.3

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator.
- **BEYOND-USE DATE:** NMT 7 days after the date on which it was compounded when stored in a refrigerator
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS (11)**  
USP Captopril RS

## Captopril Tablets

### DEFINITION

Captopril Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of captopril ( $C_9H_{15}NO_3S$ ).

### IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 4 mg/mL in methanol

**Sample solution:** 4 mg/mL of captopril in methanol prepared as follows. Dissolve the equivalent to 100 mg of captopril from a portion of powdered Tablets, taken in a conical flask, in 25 mL of methanol. Stir for 30 min using a magnetic stirrer, and centrifuge. Use the clear supernatant.

#### Chromatographic system

**Application volume:** 50 µL as streaks

**Developing solvent system:** Toluene, methanol, and glacial acetic acid (75:1:25)

**Spray reagent:** Freshly prepared mixture of 1 volume of ammonium hydroxide and 6 volumes of a solution of 0.04% 5,5'-dithiobis(2-nitrobenzoic acid) in methanol

**Analysis:** Proceed as directed in the chapter. Locate the spots on the plate by lightly spraying with *Spray reagent*.

Acceptance criteria: Meet the requirements

### ASSAY

#### • PROCEDURE

Protect solutions from exposure to air. Use within 8 h of preparation.

**Mobile phase:** 550 mL of methanol and 450 mL of water containing 0.50 mL of phosphoric acid

**Standard solution:** 1 mg/mL of USP Captopril RS and 0.05 mg/mL of USP Captopril Disulfide RS in *Mobile phase*

**Sample solution:** Nominally equivalent to 1 mg/mL of captopril prepared as follows. To NLT 20 Tablets in a suitable volumetric flask add *Mobile phase* to fill about 50% of the volume of the flask, and sonicate for 15 min. Dilute with *Mobile phase* to volume, shake by mechanical means for 15 min, and filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1 with about 15% hydrocarbon load

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for captopril and captopril disulfide are 0.5 and 1.0, respectively.]



**Suitability requirements**

**Resolution:** NLT 2.0 between captopril and captopril disulfide

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of captopril ( $C_9H_{15}NO_3S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of captopril from the *Sample solution*

$r_S$  = peak response of captopril from the *Standard solution*

$C_S$  = concentration of USP Captopril RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of captopril in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION (711)**

[NOTE—Completely deaerate the *Medium* to minimize exposure of captopril to air, and analyze the samples immediately.]

**Medium:** 0.01 N hydrochloric acid; 900 mL

**Apparatus 1:** 50 rpm

**Time:** 20 min

**Detector:** UV 205 nm

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration similar to that of a known concentration of USP Captopril RS in *Medium*.

**Tolerances:** NLT 80% (Q) of the labeled amount of captopril ( $C_9H_{15}NO_3S$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**IMPURITIES**• **LIMIT OF CAPTOPRIL DISULFIDE**

Protect solutions from exposure to air. Use within 8 h of preparation.

**Mobile phase and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability solution:** Use the *Standard solution* prepared as directed in the *Assay*.

**Standard solution:** 0.05 mg/mL of USP Captopril Disulfide RS in *Mobile phase*

**Sample solution:** Nominally 1 mg/mL of captopril in *Mobile phase* prepared as follows. Transfer an amount equivalent to 25 mg of captopril from NLT 20 finely powdered Tablets to a suitable centrifuge tube. Add 25 mL of *Mobile phase*, sonicate for 15 min, and centrifuge. Use the clear supernatant.

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for captopril and captopril disulfide are 0.5 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between captopril and captopril disulfide, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of captopril disulfide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of captopril disulfide from the *Sample solution*

$r_S$  = peak response of captopril disulfide from the *Standard solution*

$C_S$  = concentration of USP Captopril Disulfide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of captopril in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 3.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Captopril RS

USP Captopril Disulfide RS

L-Proline, 1,1'-[dithiobis(2-methyl-1-oxo-3,1-propanediyl)]bis-[S-(R\*,R\*)]-

$C_{18}H_{28}N_2O_6S_2$  432.55

**Captopril and Hydrochlorothiazide Tablets****DEFINITION**

Captopril and Hydrochlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of captopril ( $C_9H_{15}NO_3S$ ) and hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ).

**IDENTIFICATION**

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Methanol, phosphoric acid, and water (250: 0.5: 750)

**System suitability solution:** 0.3 mg/mL each of USP Captopril RS, USP Hydrochlorothiazide RS, and USP Benzothiadiazine Related Compound A RS in *Mobile phase*

**Standard solution:** 0.3 mg/mL of USP Hydrochlorothiazide RS and about 0.3/ mg/mL of USP Captopril RS in *Mobile phase*, *J* being the ratio of the labeled amount of captopril (mg) to the labeled amount of hydrochlorothiazide (mg) per Tablet

**Sample solution:** Nominally equivalent to 0.3 mg/mL of hydrochlorothiazide prepared as follows. Transfer an amount equivalent to 15 mg of hydrochlorothiazide from NLT 20 finely powdered Tablets to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, sonicate for 15 min with occasional shaking, and centrifuge.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 30-cm; packing L11

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for benzothiadiazine related compound A, hydrochlorothiazide, and captopril are 0.4, 0.5, and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.7 between the void volume and benzothiadiazine related compound A, NLT 1.8 between benzothiadiazine related compound A and hydrochlorothiazide, and NLT 2.0 between captopril and hydrochlorothiazide; *System suitability solution*

**Relative standard deviation:** NMT 3.0%, *Standard solution*



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amounts of captopril ( $C_9H_{15}NO_3S$ ) and hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of captopril or hydrochlorothiazide from the *Sample solution*  
 $r_S$  = peak response of captopril or hydrochlorothiazide from the *Standard solution*  
 $C_S$  = concentration of USP Captopril RS or USP Hydrochlorothiazide RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of captopril or hydrochlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION** (711)

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 50 rpm

**Times:** 20 min for captopril; 30 min for hydrochlorothiazide

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration similar to that of known concentrations of USP Captopril RS and USP Hydrochlorothiazide RS in *Medium*.

**Chromatographic system, System suitability, and Analysis:** Proceed as directed in the *Assay*.

**Tolerances:** NLT 80% (Q) of the labeled amount of captopril ( $C_9H_{15}NO_3S$ ) and NLT 60% (Q) of the labeled amount of hydrochlorothiazide ( $C_7H_8ClN_3O_4S_2$ ) are dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements**IMPURITIES**• **LIMIT OF CAPTOPRIL DISULFIDE**

**Mobile phase:** Methanol, phosphoric acid, and water (450:0.5:550)

**System suitability solution:** 0.0075 mg/mL each of USP Captopril RS and USP Hydrochlorothiazide RS, and 0.015 mg/mL of USP Captopril Disulfide RS in *Mobile phase*

**Standard solution:** 15 µg/mL of USP Captopril Disulfide RS in *Mobile phase*

**Sample solution:** Nominally equivalent to 0.5 mg/mL of captopril prepared as follows. Transfer an amount equivalent to 25 mg of captopril from NLT 20 finely powdered Tablets to a 50-mL volumetric flask, add about 20 mL of *Mobile phase*, and sonicate for 15 min with occasional shaking. Dilute with *Mobile phase* to volume, and centrifuge. Use the clear supernatant.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** Packing L11

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for captopril and captopril disulfide are 0.3 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 4.0 between captopril and captopril disulfide. Both peaks are resolved from hydrochlorothiazide, *System suitability solution*.

**Relative standard deviation:** NMT 3.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of captopril disulfide in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of captopril disulfide from the *Sample solution*  
 $r_S$  = peak response of captopril disulfide from the *Standard solution*  
 $C_S$  = concentration of USP Captopril Disulfide RS in the *Standard solution* (µg/mL)  
 $C_U$  = nominal concentration of captopril in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 3.0%

• **LIMIT OF BENZOTHIADIAZINE RELATED COMPOUND A**

**Mobile phase, System suitability solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** 10 µg/mL of USP Benzothiadiazine Related Compound A RS in *Mobile phase*

**Analysis**

**Samples:** *Sample solution* and *Standard solution*  
Calculate the percentage of benzothiadiazine related compound A in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of benzothiadiazine related compound A from the *Sample solution*  
 $r_S$  = peak response of benzothiadiazine related compound A from the *Standard solution*  
 $C_S$  = concentration of USP Benzothiadiazine Related Compound A RS in the *Standard solution* (µg/mL)  
 $C_U$  = nominal concentration of hydrochlorothiazide in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 1.0%

**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers.• **USP REFERENCE STANDARDS** (11)

USP Benzothiadiazine Related Compound A RS

4-Amino-6-chloro-1,3-benzenedisulfonamide.

$C_6H_8ClN_3O_4S_2$  285.73

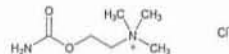
USP Captopril RS

USP Captopril Disulfide RS

L-Proline, 1,1'-[dithiobis(2-methyl-1-oxo-3,1-propanediyl)]bis-[S-(R\*,R\*)]-.

$C_{18}H_{28}N_2O_6S_2$  432.55

USP Hydrochlorothiazide RS

**Carbachol**

$C_6H_{15}ClN_2O_2$

182.65

Ethanaminium, 2-[(aminocarbonyl)oxy]-N,N,N-trimethyl-, chloride;

Choline chloride, carbamate [51-83-2].

**DEFINITION**

Carbachol contains NLT 99.0% and NMT 101.0% of carbachol ( $C_6H_{15}ClN_2O_2$ ), calculated on the dried basis.



**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL, Chloride** (191)  
Sample solution: Carbachol in water (1 in 20)  
Acceptance criteria: Meets the requirements of the silver nitrate precipitate test

**ASSAY**• **PROCEDURE**

**Sample:** 150 mg of Carbachol  
**Titrimetric system**  
**Mode:** Direct titration  
**Titrant:** 0.1 N perchloric acid VS  
**Endpoint detection:** Potentiometric  
**Blank:** 10 mL of glacial acetic acid TS and 40 mL of acetic anhydride  
**Analysis:** Dissolve the *Sample* in a mixture of 10 mL of glacial acetic acid TS and 40 mL of acetic anhydride. Titrate with *Titrant*. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 18.27 mg of carbachol ( $C_6H_{15}ClN_2O_2$ ).  
**Acceptance criteria:** 99.0%–101.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **ORDINARY IMPURITIES** (466)  
**Solvent:** Methanol and water (80:20)  
**Eluant:** Alcohol  
**Visualization:** 17  
**Analysis**  
**Samples:** *Test Solution* and *Standard Solutions*  
Proceed as directed in the chapter except use a thin-layer chromatographic plate coated with aluminum oxide.  
**Acceptance criteria:** Meets the requirements

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE** (741): 200°–204°, with some decomposition
- **LOSS ON DRYING** (731)  
**Sample:** 1 g of Carbachol  
**Analysis:** Dry the *Sample* at 105° for 2 h.  
**Acceptance criteria:** NMT 2.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Carbachol RS

**Solution B:** *Solution A* and 1 N sodium hydroxide (50:50). Prepare fresh daily.

**Solution C:** Phenol in water (1 in 200)

**Solution D:** Potassium iodide in water (3 in 1000)

**Standard solution:** 100 µg/mL of USP Carbachol RS in water

**Sample solution:** Nominally 100 µg/mL of carbachol from a volume of *Intraocular Solution* in water

**Instrumental conditions**

**Mode:** UV-Vis

**Analytical wavelength:** Maximum absorbance at about 590 nm

**Cell:** 1 cm

**Blank:** Water

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
Transfer 2.0-mL portions each of the *Standard solution*, *Sample solution*, and *Blank* to separate 50-mL conical flasks. To each flask add 1.0 mL of 0.1 N hydrochloric acid. Treat each as follows. Add 4.0 mL of *Solution B*, rinsing the inner walls of the flask with small portions of water, mix, and allow to stand for 15 min, accurately timed. Add 2.0 mL of *Solution C*, rinsing the walls of the flask with *Solution C* and with additional small portions of water. Mix, and allow to stand for 5 min. Add 2.0 mL of 3.5 N hydrochloric acid, washing the sides of the flask upon addition. Rinse the flask sparingly with 0.1 N hydrochloric acid to ensure complete acidification of all content, then mix. Add 1.0 mL of *Solution D*, and allow to stand for 5 min. Add 3.0 mL of starch TS, mix, and transfer the solutions to 50-mL volumetric flasks with the aid of several small portions of water, and dilute each solution with water to volume.

Concomitantly determine the absorbances of the solutions from the *Sample solution* and the *Standard solution* against the *Blank*.

Calculate the percentage of the labeled amount of carbachol ( $C_6H_{15}ClN_2O_2$ ) in the portion of *Intraocular Solution* taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Carbachol RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of carbachol in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–115.0%

**SPECIFIC TESTS**

- **STERILITY TESTS** (71): Meets the requirements
- **PH** (791): 5.0–7.5

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, at controlled room temperature, and protect from freezing.
- **LABELING:** Label it to indicate that it is for single-dose intraocular use only, and that the unused portion is to be discarded.
- **USP REFERENCE STANDARDS** (11)  
USP Carbachol RS

**Carbachol Intraocular Solution****DEFINITION**

Carbachol Intraocular Solution is a sterile solution of Carbachol in an aqueous medium. It contains NLT 90.0% and NMT 115.0% of the labeled amount of carbachol ( $C_6H_{15}ClN_2O_2$ ). It contains no preservatives or antimicrobial agents.

**IDENTIFICATION**• **A.**

**Solution A:** A saturated (filtered) ammonium reineckate solution

**Analysis:** To 5 mL of *Intraocular Solution* add 4–5 drops of *Solution A*.

**Acceptance criteria:** A pink precipitate is formed that is soluble in acetone; the acetone solution is red.

**ASSAY**• **PROCEDURE**

**Solution A:** Dilute 1 volume of sodium hypochlorite TS with water to 15 volumes, and allow to stand for 30 min.

**Carbachol Ophthalmic Solution****DEFINITION**

Carbachol Ophthalmic Solution is a sterile solution of Carbachol in an isotonic, aqueous medium. It contains NLT 95.0% and NMT 105.0% of the labeled amount of carbachol ( $C_6H_{15}ClN_2O_2$ ). It may contain suitable preservatives and antimicrobial agents.



**IDENTIFICATION**• **A.**

**Solution A:** Ammonium reineckate in water (1 in 30)

**Sample solution:** 1 mg/mL of carbachol from Ophthalmic Solution in water

**Analysis:** Add 5 mL of *Solution A* to the *Sample solution*, and shake vigorously for 1 min.

**Acceptance criteria:** A red precipitate is formed; it is soluble in acetone.

**ASSAY**• **PROCEDURE**

**Solution A:** Dilute 1 volume of sodium hypochlorite TS with water to 15 volumes, and allow to stand for 30 min.

**Solution B:** *Solution A* and 1 N sodium hydroxide (50:50). Prepare fresh daily.

**Solution C:** Phenol in water (1 in 200)

**Solution D:** Potassium iodide in water (3 in 1000)

**Standard solution:** 100 µg/mL of USP Carbachol RS in water

**Sample solution:** Nominally 100 µg/mL of carbachol from a volume of Ophthalmic Solution in water

**Instrumental conditions**

**Mode:** UV-Vis

**Analytical wavelength:** Maximum absorbance at about 590 nm

**Cell:** 1 cm

**Blank:** Water

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
Transfer 2.0-mL portions each of the *Standard solution*, *Sample solution*, and *Blank* to separate 50-mL conical flasks. To each flask add 1.0 mL of 0.1 N hydrochloric acid. Treat each as follows. Add 4.0 mL of *Solution B*, rinsing the inner walls of the flask with small portions of water, mix, and allow to stand for 15 min, accurately timed. Add 2.0 mL of *Solution C*, rinsing the walls of the flask with *Solution C* and with additional small portions of water. Mix, and allow to stand for 5 min. Add 2.0 mL of 3.5 N hydrochloric acid, washing the sides of the flask upon addition. Rinse the flask sparingly with 0.1 N hydrochloric acid to ensure complete acidification of all content, then mix. Add 1.0 mL of *Solution D*, and allow to stand for 5 min. Add 3.0 mL of starch TS, mix, and transfer the solutions to 50-mL volumetric flasks with the aid of several small portions of water, and dilute each solution with water to volume.

Concomitantly determine the absorbances of the solutions from the *Sample solution* and the *Standard solution* against the *Blank*.

Calculate the percentage of the labeled amount of carbachol ( $C_6H_{15}ClN_2O_2$ ) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Carbachol RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of carbachol in the *Sample solution* (µg/mL)

**Acceptance criteria:** 95.0%–105.0%

**SPECIFIC TESTS**

• **STERILITY TESTS (71):** Meets the requirements

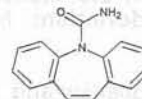
• **PH (791):** 5.0–7.0

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Carbachol RS

**Carbamazepine**

$C_{15}H_{12}N_2O$

236.27

5*H*-Dibenz[*b,f*]azepine, 5-carboxamide;

5*H*-Dibenz[*b,f*]azepine-5-carboxamide [298-46-4].

**DEFINITION**

Carbamazepine contains NLT 98.0% and NMT 102.0% of carbamazepine ( $C_{15}H_{12}N_2O$ ), calculated on the dried basis.

**IDENTIFICATION**

• **A. INFRARED ABSORPTION (197M)**

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Solution A:** Add 0.5 mL of triethylamine and 0.5 mL of formic acid to 1000 mL of water.

**Solution B:** Add 0.25 mL of formic acid to 1000 mL of methanol.

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0.0	80	20
3.0	80	20
12.0	60	40
18.0	45	55
20.0	45	55
20.1	80	20
23.0	80	20

**Diluent:** Methanol and water (50:50)

**System suitability stock solution:** 0.02 mg/mL each of USP Carbamazepine RS and USP Carbamazepine Related Compound A RS prepared as follows. First dissolve the Reference Standards in 50% of the final flask volume of methanol, then dilute with water to volume.

**System suitability solution:** 0.002 mg/mL each of USP Carbamazepine RS and USP Carbamazepine Related Compound A RS from *System suitability stock solution* in *Diluent*

**Standard solution:** 0.1 mg/mL of USP Carbamazepine RS prepared as follows. First dissolve the Reference Standard in 50% of the final flask volume of methanol, then dilute with water to volume.

**Sample solution:** 0.1 mg/mL of Carbamazepine prepared as follows. First dissolve the sample in 50% of the final flask volume of methanol, then dilute with water to volume. Pass through a suitable filter of 0.2-µm pore size.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 2.1-mm × 10-cm; 1.8-μm packing L10

Column temperature: 40°

Flow rate: 0.3 mL/min

Injection volume: 2 μL

**System suitability**Samples: *System suitability solution* and *Standard solution***Suitability requirements**Resolution: NLT 1.7 between carbamazepine related compound A and carbamazepine, *System suitability solution*Tailing factor: NMT 2.0, *Standard solution*Relative standard deviation: NMT 0.73%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of carbamazepine (C<sub>15</sub>H<sub>12</sub>N<sub>2</sub>O) in the portion of Carbamazepine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

**IMPURITIES**• **CHLORIDE AND SULFATE, Chloride (221)**

Sample solution: Boil 1.0 g of Carbamazepine in 20.0 mL of water for 10 min, cool, adjust the volume to 20 mL, and filter. Use a 10.0-mL portion of the filtrate.

Acceptance criteria: 0.014%; the *Sample solution* contains no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid.**Delete the following:**• **HEAVY METALS, Method II (231):** NMT 10 ppm • (Official 1-

Jan-2018)

• **RESIDUE ON IGNITION (281)**

Sample: 2.0 g of Carbamazepine

Acceptance criteria: NMT 0.1%

• **ORGANIC IMPURITIES**Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system: Proceed as directed in the *Assay*.

Standard stock solution: 0.02 mg/mL each of USP Carbamazepine RS, USP Carbamazepine Related Compound A RS, and USP Carbamazepine Related Compound B RS, prepared as follows. First dissolve the Reference Standards in 50% of the final flask volume of methanol, then dilute with water to volume.

Standard solution: 0.002 mg/mL each of USP Carbamazepine RS, USP Carbamazepine Related Compound A RS, and USP Carbamazepine Related Compound B RS from *Standard stock solution* in *Diluent*.

Sample solution: 1.0 mg/mL of Carbamazepine prepared as follows. First dissolve the sample in 50% of the final flask volume of methanol, then dilute with water to volume. Pass through a suitable filter of 0.2-μm pore size.

**System suitability**Sample: *Standard solution***Suitability requirements**

Resolution: NLT 1.7 between carbamazepine related compound A and carbamazepine

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Carbamazepine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each specified impurity from the *Sample solution* $r_S$  = peak response of the corresponding USP Reference Standard from the *Standard solution* $C_S$  = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL) $C_U$  = concentration of Carbamazepine in the *Sample solution* (mg/mL)

Calculate the percentage of each individual unspecified impurity in the portion of Carbamazepine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each unspecified impurity from the *Sample solution* $r_S$  = peak response of carbamazepine from the *Standard solution* $C_S$  = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Carbamazepine in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2. Disregard any impurity peak less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carbamazepine related compound A <sup>a</sup>	0.96	0.2
Carbamazepine	1.00	—
Carbamazepine related compound B <sup>b</sup>	1.45	0.2
Individual unspecified impurity	—	0.2
Total impurities	—	0.5

<sup>a</sup> 10,11-Dihydrocarbamazepine.<sup>b</sup> 5H-Dibenz[b,f]azepine.**SPECIFIC TESTS**• **ACIDITY**

Sample solution: 50 mg/mL of Carbamazepine in water prepared as follows. Mix 2.0 g of Carbamazepine in 40.0 mL of water for 15 min, and filter through paper.

Analysis: To a 10.0-mL aliquot of *Sample solution* add 1 drop of phenolphthalein TS, and titrate with 0.01 N sodium hydroxide VS. Perform a blank determination, and make any necessary correction.

Acceptance criteria: NMT 1.0 mL of 0.01 N sodium hydroxide VS is required for each 1.0 g of Carbamazepine.

• **ALKALINITY**

Sample solution: 50 mg/mL of Carbamazepine in water prepared as follows. Mix 2.0 g of Carbamazepine in 40.0 mL of water for 15 min, and filter through paper.

Analysis: To a 10.0-mL aliquot of *Sample solution* add 1 drop of methyl red TS, and titrate with 0.01 N hydrochloric acid VS. Perform a blank determination, and make any necessary correction.

Acceptance criteria: NMT 1.0 mL of 0.01 N hydrochloric acid VS is required for each 1.0 g of Carbamazepine.



- **LOSS ON DRYING** (731)  
Analysis: Dry at 105° for 2 h.  
Acceptance criteria: NMT 0.5%
- **X-RAY DIFFRACTION** (941): The X-ray diffraction pattern conforms to that of USP Carbamazepine RS, similarly determined.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Carbamazepine RS  
USP Carbamazepine Related Compound A RS  
10,11-Dihydrocarbamazepine.  
 $C_{15}H_{14}N_2O$  238.28  
USP Carbamazepine Related Compound B RS  
5H-Dibenz[b,f]azepine.  
 $C_{14}H_{11}N$  193.24

## Carbamazepine Oral Suspension

#### DEFINITION

Carbamazepine Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ).

#### IDENTIFICATION

##### A. INFRARED ABSORPTION (197S)

**Sample solution:** Place 5 mL of Oral Suspension in a separator containing 20 mL of 0.1 N sodium hydroxide, and extract with 25 mL of chloroform. Pass the extract through anhydrous sodium sulfate supported on filter paper into a beaker. Wash the anhydrous sodium sulfate with 10 mL of chloroform, and add the washing to the extract. Evaporate the chloroform extract to dryness with the aid of a stream of nitrogen. Dissolve the residue in 10 mL of methylene chloride.

**Acceptance criteria:** Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Solution A:** Add 0.5 mL of triethylamine and 0.5 mL of formic acid to 1000 mL of water.

**Solution B:** Add 0.25 mL of formic acid to 1000 mL of methanol.

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0.0	80	20
3.0	80	20
12.0	60	40
18.0	45	55
20.0	45	55
20.1	80	20
23.0	80	20

**Diluent:** Methanol

**System suitability solution:** 0.002 mg/mL each of USP Carbamazepine RS and USP Carbamazepine Related Compound A RS in *Diluent*

**Standard solution:** 0.1 mg/mL of USP Carbamazepine RS in *Diluent*

**Sample solution:** Nominally 0.1 mg/mL of carbamazepine from a volume of Oral Suspension prepared as follows. Weigh and transfer freshly mixed Oral Suspension equivalent to 20 mg of carbamazepine to a 200-mL vol-

umetric flask. Add about 140 mL of *Diluent*, shake by mechanical means for about 30 min, sonicate for about 2 min, and dilute with *Diluent* to volume. Pass through a suitable filter of 0.2- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 2.1-mm  $\times$  10-cm; 1.8- $\mu$ m packing L10

**Column temperature:** 40°

**Flow rate:** 0.3 mL/min

**Injection volume:** 2  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.7 between carbamazepine related compound A and carbamazepine, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements for oral suspension packaged in single-unit containers
- **DELIVERABLE VOLUME** (698): Meets the requirements for oral suspension packaged in multiple-unit containers

#### IMPURITIES

##### ORGANIC IMPURITIES

**Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.002 mg/mL each of USP Carbamazepine RS, USP Carbamazepine Related Compound A RS, and USP Carbamazepine Related Compound B RS in *Diluent*

**Sample solution:** Nominally 1.0 mg/mL of carbamazepine from a volume of Oral Suspension prepared as follows. Weigh and transfer freshly mixed Oral Suspension equivalent to 50 mg of carbamazepine to a 50-mL volumetric flask. Add about 35 mL of *Diluent*, shake by mechanical means for about 30 min, sonicate for about 2 min, dilute with *Diluent* to volume, and shake for about 5 min. Pass through a suitable filter of 0.2- $\mu$ m pore size.

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.7 between carbamazepine related compound A and carbamazepine

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carbamazepine related compound B in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response of carbamazepine related compound B from the *Sample solution*  
 $r_S$  = peak response of carbamazepine related compound B from the *Standard solution*  
 $C_S$  = concentration of USP Carbamazepine Related Compound B RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)  
 Calculate the percentage of each individual unspecified impurity in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of each unspecified impurity from the *Sample solution*  
 $r_S$  = peak response of carbamazepine from the *Standard solution*  
 $C_S$  = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)  
**Acceptance criteria:** See Table 2. Disregard any impurity peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carbamazepine related compound A <sup>a,b</sup>	0.96	—
Carbamazepine	1.00	—
Carbamazepine related compound B <sup>c</sup>	1.45	0.2
Individual unspecified impurity	—	0.2
Total impurities	—	0.5

<sup>a</sup> 10,11-Dihydrocarbamazepine.

<sup>b</sup> Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

<sup>c</sup> 5H-Dibenz[b,f]azepine.

### SPECIFIC TESTS

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total bacterial count does not exceed  $1 \times 10^2$  cfu/g, and the tests for *Salmonella* species and *Escherichia coli* are negative.

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, protected from freezing and from excessive heat.
- USP REFERENCE STANDARDS (11)**
  - USP Carbamazepine RS
  - USP Carbamazepine Related Compound A RS
  - 10,11-Dihydrocarbamazepine.  
 $C_{15}H_{14}N_2O$  238.28
  - USP Carbamazepine Related Compound B RS
  - 5H-Dibenz[b,f]azepine.  
 $C_{14}H_{11}N$  193.24

## Carbamazepine Tablets

### DEFINITION

Carbamazepine Tablets contain NLT 92.0% and NMT 108.0% of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ).

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197M)

**Sample solution:** Nominally 250 mg of carbamazepine from powdered Tablets in 15 mL of acetone

**Analysis:** Boil the *Sample solution* for 5 min in a suitable beaker. Filter while hot, using two 5-mL portions of hot acetone to effect transfer. Evaporate the filtrate with the aid of nitrogen to 5 mL, and cool in an ice bath until crystals are formed. Filter the crystals, wash with 3 mL of cold acetone, and dry under vacuum at 70° for 30 min. Use the crystals.

**Acceptance criteria:** Meet the requirements

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Mobile phase:** Methanol, tetrahydrofuran, and water (12:3:85). Add 0.22 mL of formic acid and then 0.5 mL of triethylamine to each L.

**Diluent:** Methanol and water (50:50)

**System suitability stock solution:** 0.1 mg/mL of USP Carbamazepine RS and 0.5 mg/mL of USP Carbamazepine Related Compound A RS in methanol. Sonication may be used to aid in dissolution.

**System suitability solution:** 0.01 mg/mL of USP Carbamazepine RS and 0.05 mg/mL of USP Carbamazepine Related Compound A RS from *System suitability stock solution* in *Diluent*

**Standard stock solution:** 2 mg/mL of USP Carbamazepine RS in methanol. Sonication may be used to aid in dissolution.

**Standard solution:** 0.2 mg/mL of USP Carbamazepine RS from *Standard stock solution* in *Diluent*

**Sample stock solution:** Nominally 2 mg/mL of carbamazepine from NLT 20 Tablets prepared as follows. Finely powder the Tablets, and transfer a portion of the powder to a suitable volumetric flask. Add 80% of the final flask volume of methanol, sonicate for 15 min, and allow to cool to room temperature. Dilute with methanol to volume. Pass through a suitable filter and discard the first few mL of filtrate.

**Sample solution:** Nominally 0.2 mg/mL of carbamazepine from *Sample stock solution* in *Diluent*

#### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.0- or 4.6-mm  $\times$  25-cm; 7- $\mu$ m packing L10

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**Run time:** NLT 1.6 times the retention time of carbamazepine

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 3 for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.7 between carbamazepine related compound A and carbamazepine, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*



- $C_s$  = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)  
 Acceptance criteria: 92.0%–108.0%

**PERFORMANCE TESTS****• DISSOLUTION (711)**

For products labeled as 100-mg chewable Tablets

**Test 1:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

**Medium:** Water containing 1% sodium lauryl sulfate; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 60 min

**Standard solution:** USP Carbamazepine RS in *Medium*. [NOTE—A volume of methanol NMT 1% of the final total volume of the *Standard solution* may be used to dissolve the carbamazepine.]

**Sample solution:** Filtered portion of the solution under test, diluted with *Medium* if necessary

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** Maximum absorbance at about 288 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ) dissolved:

$$\text{Result} = (A_u/A_s) \times C_s \times V \times (1/L) \times 100$$

$A_u$  = absorbance of the *Sample solution*

$A_s$  = absorbance of the *Standard solution*

$C_s$  = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% ( $Q$ ) of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ) is dissolved. Use *Dissolution (711)*, *Acceptance Table 1* with the following exceptions: at  $S_2$ , no unit is less than  $Q - 5\%$ ; at  $S_3$ , no unit is less than  $Q - 10\%$ ; and NMT 2 of the 24 units are less than  $Q - 5\%$ .

For products labeled as 200-mg Tablets

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium, Apparatus 2, Standard solution, Sample solution, and Instrumental conditions:** Proceed as directed in *Test 1*.

**Times:** 15 and 60 min

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the concentration ( $C_i$ ) of carbamazepine ( $C_{15}H_{12}N_2O$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result} = (A_u/A_s) \times C_s$$

$A_u$  = absorbance of the *Sample solution*

$A_s$  = absorbance of the *Standard solution*

$C_s$  = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V_2) + (C_1 \times V_3)] \times (1/L) \times 100$$

$C_i$  = concentration of carbamazepine in the portion of sample withdrawn at the specified time point ( $i$ ) (mg/mL)

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

$V_2$  = volume of the *Medium* at time point 2 (mL)

$V_3$  = volume of the *Sample solution* withdrawn at each time point ( $i$ ) (mL)

**Tolerances:** See *Table 1*.

**Table 1**

Time Point ( $i$ )	Time (min)	Amount Dissolved (%)
1	15	45–75
2	60	NLT 75

Use *Dissolution (711)*, *Acceptance Table 2* with the following exceptions. At 15 min: at  $L_2$ , no unit is more than 5% outside the stated range; at  $L_3$ , no unit is more than 10% outside the stated range; and NMT 2 of the 24 units are more than 5% outside the stated range. At 60 min: at  $L_2$ , no unit is less than  $Q - 5\%$ ; at  $L_3$ , no unit is less than  $Q - 10\%$ ; and NMT 2 of the 24 units are less than  $Q - 5\%$ .

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium, Apparatus 2, Standard solution, Sample solution, and Instrumental conditions:** Proceed as indicated in *Test 1*.

**Times:** 15 and 60 min

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the concentration ( $C_i$ ) of carbamazepine ( $C_{15}H_{12}N_2O$ ) in the sample withdrawn from the vessel at each time point ( $i$ ):

$$\text{Result} = (A_u/A_s) \times C_s$$

$A_u$  = absorbance of the *Sample solution*

$A_s$  = absorbance of the *Standard solution*

$C_s$  = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V_2) + (C_1 \times V_3)] \times (1/L) \times 100$$

$C_i$  = concentration of carbamazepine in the portion of sample withdrawn at the specified time point ( $i$ ) (mg/mL)

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

$V_2$  = volume of the *Medium* at time point 2 (mL)

$V_3$  = volume of the *Sample solution* withdrawn at each time point ( $i$ ) (mL)

**Tolerances:** See *Table 2*.

**Table 2**

Time Point ( $i$ )	Time (min)	Amount Dissolved (%)
1	15	60–85
2	60	NLT 75

Use *Dissolution (711)*, *Acceptance Table 2* with the following exceptions. At 15 min: at  $L_2$ , no unit is more than 5% outside the stated range; at  $L_3$ , no unit is more than 10% outside the stated range; and NMT 2 of the 24 units are more than 5% outside the stated range. At 60 min: at  $L_2$ , no unit is less than  $Q - 5\%$ ; at  $L_3$ , no unit is less than  $Q - 10\%$ ; and NMT 2 of the 24 units are less than  $Q - 5\%$ .

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements



## IMPURITIES

## • ORGANIC IMPURITIES

**Mobile phase, Diluent, and System suitability solution:** Proceed as directed in the Assay.

**Standard stock solution:** 0.02 mg/mL each of USP Carbamazepine RS, USP Carbamazepine Related Compound B RS, and USP 9-Methylacridine RS in methanol. Sonication may be used to aid in dissolution.

**Standard solution:** 0.001 mg/mL each of USP Carbamazepine RS, USP Carbamazepine Related Compound B RS, and USP 9-Methylacridine RS from *Standard stock solution* in *Diluent*

**Sample solution:** Nominally 1 mg/mL of carbamazepine from NLT 20 Tablets prepared as follows. Finely powder the Tablets, and transfer a portion of the powder to a suitable volumetric flask. Add about 50% of the final flask volume of *Diluent*, sonicate for 15 min, and allow to cool to room temperature. Dilute with *Diluent* to volume. Pass through a suitable filter and discard the first few mL of filtrate.

**Chromatographic system:** Proceed as directed in the Assay except use a *Run time* of NLT 3.5 times the retention time of carbamazepine.

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 3 for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.7 between carbamazepine related compound A and carbamazepine, *System suitability solution*

**Relative standard deviation:** NMT 10.0% each for carbamazepine, carbamazepine related compound B, and 9-methylacridine, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of carbamazepine related compound B and 9-methylacridine in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of carbamazepine related compound B or 9-methylacridine from the *Sample solution*  
 $r_s$  = peak response of carbamazepine related compound B or 9-methylacridine from the *Standard solution*  
 $C_s$  = concentration of USP Carbamazepine Related Compound B RS or USP 9-Methylacridine RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

Calculate the percentage of other degradation products in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of any individual unspecified degradation product from the *Sample solution*  
 $r_s$  = peak response of carbamazepine from the *Standard solution*  
 $C_s$  = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 3. Disregard peaks below 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
9-Methylacridine	0.54	0.2
Carbamazepine related compound A*	0.87	—
Carbamazepine	1.0	—
Carbamazepine related compound B	3.1	0.2
Any individual unspecified degradation product	—	0.2
Total degradation products	—	0.30

\* This is a process impurity that is controlled in the drug substance. It is not to be reported or included in the total degradation products.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably of glass. Protect from light and moisture. Store at controlled room temperature.
- **LABELING:** The labeling indicates the *Dissolution* test with which the product complies.
- **USP REFERENCE STANDARDS (11)**
  - USP Carbamazepine RS
  - USP Carbamazepine Related Compound A RS
  - 10,11-Dihydrocarbamazepine.  
 $C_{15}H_{14}N_2O$  238.28
  - USP Carbamazepine Related Compound B RS
  - 5H-Dibenz[*b,f*]azepine.  
 $C_{14}H_{11}N$  193.24
  - USP 9-Methylacridine RS
  - 9-Methylacridine.  
 $C_{14}H_{11}N$  193.24

**Carbamazepine Extended-Release Tablets****DEFINITION**

Carbamazepine Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ).

**IDENTIFICATION**• **A. ULTRAVIOLET ABSORPTION (197U)**

**Standard solution:** 10 µg/mL of USP Carbamazepine RS in methanol

**Sample solution:** Finely powder 1 Tablet, and quantitatively transfer the powder, with the aid of methanol, to a 100-mL volumetric flask. Add about 70 mL of methanol, and shake by mechanical means for 60 min. Sonicate for 15 min, and dilute with methanol to volume. Allow to stand for 10–15 min. Dilute a portion of the clear solution with methanol to obtain a solution containing about 10 µg/mL of carbamazepine.

**Acceptance criteria:** Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Methanol, methylene chloride, and water (450:45:600)

**Internal standard solution:** 600 µg/mL of phenytoin in methanol

**Standard stock solution:** 200 µg/mL of USP Carbamazepine RS in methanol

**Standard solution:** 100 µg/mL of carbamazepine from *Standard stock solution* in *Internal standard solution*

**System suitability solution:** 50 µg/mL of carbamazepine from *Standard solution* in *Internal standard solution*



**Sample stock solution A:** Nominally 4 mg/mL of carbamazepine from finely powdered Tablets prepared as follows. Finely powder 10 Tablets. Transfer the powder to an appropriate volumetric flask with the aid of methanol. Add 70% of the flask volume of methanol. Shake by mechanical means for 60 min. Sonicate for 15 min, and dilute with methanol to volume. Allow to stand for 10–15 min, and then filter a portion of the supernatant. Use the clear filtrate.

**Sample stock solution B:** Nominally 0.2 mg/mL of carbamazepine from *Sample stock solution A* in methanol

**Sample solution:** Nominally 100 µg/mL of carbamazepine from *Sample stock solution B* in *Internal standard solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Columns**

**Guard:** 4.6-mm × 30-mm; 7-µm packing L7

**Analytical:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for phenytoin and carbamazepine are about 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.8 between phenytoin and carbamazepine

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of carbamazepine to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of carbamazepine to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Carbamazepine RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of carbamazepine in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium**

**For Tablets labeled to contain 100 mg or 200 mg:**

Water; 900 mL

**For Tablets labeled to contain 400 mg:** Water;

1800 mL

**Apparatus 1:** 100 rpm

**Times:** 3, 6, 12, and 24 h

**Standard solution:** USP Carbamazepine RS in *Medium*

**Sample solution:** Filtered portions of the solution under test, diluted with *Medium* if necessary

#### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** The wavelength of maximum absorbance at about 284 nm

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ) dissolved at each time using the UV absorption.

**Tolerances:** See *Table 1*.

**Table 1**

Time (h)	Amount Dissolved
3	10%–35%
6	35%–65%
12	65%–90%
24	NLT 75%

The percentages (Q) of the labeled amount of carbamazepine ( $C_{15}H_{12}N_2O$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES: PROCEDURE 1

**Mobile phase:** Methanol, methylene chloride, and water (45:45:600)

**System suitability solution:** 60 µg/mL of phenytoin and 20 µg/mL of USP Carbamazepine RS in methanol

**Standard solution:** 4 µg/mL of USP Carbamazepine RS in methanol

**Sample solution:** Use *Sample stock solution A* from the *Assay*.

**Chromatographic system and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of carbamazepine from the *Standard solution*

$C_S$  = concentration of USP Carbamazepine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carbamazepine in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Any individual unspecified degradation product:** NMT 0.2%

##### • ORGANIC IMPURITIES: PROCEDURE 2

**Mobile phase:** Methanol, acetonitrile, and water (35:15:50)

**System suitability solution:** 12.5 µg/mL of iminostilbene and 5.0 µg/mL of USP Carbamazepine RS in methanol

**Standard solution:** 4 µg/mL of USP Carbamazepine RS in methanol

**Sample solution:** Use *Sample stock solution A* from the *Assay*.

**Chromatographic system:** Proceed as directed in the *Assay*.

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for carbamazepine and iminostilbene are about 0.3 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 10.0 between carbamazepine and iminostilbene

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*



- $r_s$  = peak response of carbamazepine from the Standard solution  
 $C_s$  = concentration of USP Carbamazepine RS in the Standard solution (mg/mL)  
 $C_u$  = nominal concentration of carbamazepine in the Sample solution (mg/mL)

**Acceptance criteria**

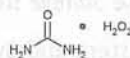
Any individual unspecified degradation product:

NMT 0.2%

Total Impurities: NMT 0.5% for all impurities from Procedure 1 and Procedure 2.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Carbamazepine RS

**Carbamide Peroxide**

$\text{CH}_6\text{N}_2\text{O}_3$  94.07

Urea, compd. with hydrogen peroxide (1:1).

Urea compound with hydrogen peroxide (1:1) [124-43-6].

» Carbamide Peroxide contains not less than 96.0 percent and not more than 102.0 percent of  $\text{CH}_6\text{N}_2\text{O}_3$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

**Identification**

A: Mix 1 mL of a solution (1 in 10) of it with 1 mL of nitric acid: a white, crystalline precipitate is formed.

B: A solution of it (1 in 10) responds to the tests for Peroxide (191).

**Assay**—Transfer about 100 mg of Carbamide Peroxide, accurately weighed, to a 500-mL iodine flask with the aid of 25 mL of water, add 5 mL of glacial acetic acid, and mix. Add 2 g of potassium iodide and 1 drop of ammonium molybdate TS, insert the stopper, and allow to stand in the dark for 10 minutes. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 4.704 mg of  $\text{CH}_6\text{N}_2\text{O}_3$ .

**Carbamide Peroxide Topical Solution**

» Carbamide Peroxide Topical Solution is a solution in anhydrous glycerin of Carbamide Peroxide or of carbamide peroxide prepared from hydrogen peroxide and Urea. It contains not less than 78.0 percent and not more than 110.0 percent, by weight, of the labeled amount of  $\text{CH}_6\text{N}_2\text{O}_3$ .

**Packaging and storage**—Preserve in tight, light-resistant containers, and avoid exposure to excessive heat.

**Identification**

A: Mix 1 mL with 1 mL of nitric acid: a white, crystalline precipitate is formed.

B: It responds to the tests for Peroxide (191).

**Specific gravity** (841): between 1.245 and 1.272.

**pH** (791): between 4.0 and 7.5.

**Assay**—Transfer an accurately weighed quantity of Topical Solution, equivalent to about 100 mg of carbamide peroxide, to a 500-mL iodine flask with the aid of 25 mL of water, add 5 mL of glacial acetic acid, and mix. Add 2 g of potassium iodide and 1 drop of ammonium molybdate TS, and allow to stand in the dark for 10 minutes. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 4.704 mg of  $\text{CH}_6\text{N}_2\text{O}_3$ .

**Carbenicillin for Injection**

» Carbenicillin for Injection contains an amount of Carbenicillin Disodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of carbenicillin ( $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_6\text{S}$ ).

**Change to read:**

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

**USP Reference standards (11)**

USP Carbenicillin Monosodium Monohydrate RS  
USP Endotoxin RS

**Identification**—It responds to the tests for Sodium (191).

**Bacterial Endotoxins Test** (85)—It contains not more than 0.05 USP Endotoxin Unit per mg of carbenicillin.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 6 g being aseptically dissolved in 200 mL of Fluid A.

**pH** (791): between 6.5 and 8.0, in the solution constituted as directed in the labeling.

**Water Determination, Method I** (921): not more than 6.0%.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions and for Labeling* (7), *Labels and Labeling for Injectable Products*.

**Change to read:****Assay**

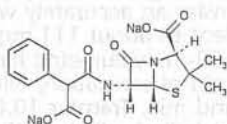
*Assay preparation 1* (where it is packaged for dispensing and where the package is represented as being a single-dose container)—Constitute Carbenicillin for Injection as directed in the labeling. Withdraw all of the withdrawable contents, and dilute quantitatively with *Buffer B.1* (CN 1-May-2017) to obtain a solution having a convenient concentration of carbenicillin.

*Assay preparation 2* (where the label states the quantity of carbenicillin in a given volume of constituted solution)—Constitute Carbenicillin for Injection as directed in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Buffer B.1* (CN 1-May-2017) to obtain a solution having a convenient concentration of carbenicillin.



**Procedure**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer B.1* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Carbenicillin Disodium



$C_{17}H_{16}N_2Na_2O_6S$  (anhydrous) 422.36  
4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[(carboxyphenylacetyl)amino]-3,3-dimethyl-7-oxo-, disodium salt, [2S-(2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ )]-  
N-(2-Carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-6-yl)-2-phenylmalonic acid disodium salt [4800-94-6].

» Carbenicillin Disodium has a potency equivalent to not less than 770  $\mu$ g of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Carbenicillin Monosodium Monohydrate RS  
USP Endotoxin RS

**Identification**—It responds to the tests for *Sodium* (191).

**pH** (791): between 6.5 and 8.0, in a solution containing 10 mg of carbenicillin per mL.

**Water Determination, Method I** (921): not more than 6.0%.

**Other requirements**—Where the label states that Carbenicillin Disodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Carbenicillin for Injection*. Where the label states that Carbenicillin Disodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Carbenicillin for Injection*.

### Change to read:

#### Assay—

**Assay preparation**—Dissolve a suitable quantity of Carbenicillin Disodium, accurately weighed, in *Buffer B.1* (CN 1-May-2017), and dilute quantitatively with *Buffer B.1* (CN 1-May-2017) to obtain a solution having a convenient concentration of carbenicillin.

**Procedure**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer B.1* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Carbenicillin Indanyl Sodium

$C_{26}H_{25}N_2NaO_6S$  516.54

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[3-[(2,3-dihydro-1H-inden-5-yl)oxy]-1,3-dioxo-2-phenylpropyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, [2S-(2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ )]-  
1-(5-Indanyl)(2S,5R,6R)-N-(2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-6-yl)-2-phenylmalonamate monosodium salt [26605-69-6].

» Carbenicillin Indanyl Sodium has a potency equivalent to not less than 630  $\mu$ g and not more than 769  $\mu$ g of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers. For periods up to 18 months, store at controlled room temperature.

**USP Reference standards** (11)—

USP Carbenicillin Indanyl Sodium RS

**Identification**—

**A: Infrared Absorption** (197K).

**B:** It responds to the tests for *Sodium* (191).

**pH** (791): between 5.0 and 8.0, in a solution containing 100 mg per mL.

**Water Determination, Method I** (921): not more than 2.0%.

#### Assay—

**Mobile phase**—Prepare a buffer of 0.0009 M tetrabutylammonium hydrogen phosphate and 0.05 M dibasic sodium phosphate as follows. Dissolve 604 mg of tetrabutylammonium phosphate and 26.8 g of dibasic sodium phosphate in 1800 mL of water, adjust with phosphoric acid to a pH of 3.8, and dilute with water to 2000 mL. Prepare a filtered and degassed mixture of this buffer and acetonitrile (116:84), allow to stand for 1 hour, and if necessary readjust with phosphoric acid to a pH of 3.8. Make any necessary adjustments (see *System Suitability* under *Chromatography* (621)).

**Diluting solvent**—Prepare a mixture of acetonitrile and 0.005 M monobasic potassium phosphate (85:15).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Carbenicillin Indanyl Sodium RS quantitatively in *Diluting solvent* to obtain a solution having a known concentration of about 250  $\mu$ g per mL. This *Standard preparation* contains the equivalent of about 222  $\mu$ g of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ) per mL.

**Assay preparation**—Transfer about 125 mg of Carbenicillin Indanyl Sodium, accurately weighed, to a 50-mL volumetric flask, dissolve in *Diluting solvent* with the aid of sonication, dilute with *Diluting solvent* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L7. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ) in each



mg of the Carbenicillin Indanyl Sodium taken by the formula:

$$0.5(CP/W)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Carbenicillin Indanyl Sodium RS, calculated on the anhydrous basis, in the *Standard preparation*; P is the assigned potency, in µg per mg, of USP Carbenicillin Indanyl Sodium RS; W is the quantity, in mg, of Carbenicillin Indanyl Sodium taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the carbenicillin indanyl peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Carbenicillin Indanyl Sodium Tablets

» Carbenicillin Indanyl Sodium Tablets contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Carbenicillin Indanyl Sodium RS

**Identification**—Triturate a quantity of finely powdered Tablets, equivalent to about 100 mg of carbenicillin, with 10 mL of a solvent mixture consisting of acetone, ethyl acetate, water, pyridine, and glacial acetic acid (200:100:75:25:1.5). Shake the mixture for 5 minutes, and dilute 1 volume of it with 9 volumes of the solvent mixture. Apply 10 µL each of this solution and of a solution of USP Carbenicillin Indanyl Sodium RS in the same solvent mixture containing 1 mg of carbenicillin per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of acetone, ethyl acetate, water, pyridine, and glacial acetic acid (400:300:75:25:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and heat the plate at 80° for 30 minutes. Allow the plate to cool, and expose it to iodine vapors in a closed chamber for about 30 seconds. Spray the plate with a reagent consisting of a 1 in 100 solution of ferric chloride in 0.1 N hydrochloric acid, potassium ferricyanide solution (1 in 100), and methanol (4:4:3): the principal spots from the test solution and the Standard solution are blue on a yellow-green background, and the  $R_F$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution ( $R_F$  about 0.5).

**Dissolution** (711)—

Medium: water; 900 mL.

Apparatus 1: 100 rpm.

Time: 45 minutes.

**Procedure**—Determine the amount of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ) equivalent dissolved from the difference between UV absorbances at the wavelengths of maximum and minimum absorbance at about 267 nm and 254 nm, respectively, of filtered portions of the solution under test, suitably diluted with water, in comparison with a Standard solution having a known concentration of USP Carbenicillin Indanyl Sodium RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{17}H_{18}N_2O_6S$  equivalent is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water Determination, Method I** (921): not more than 2.0%.

**Assay**—

*Mobile phase, Diluting solvent, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Carbenicillin Indanyl Sodium.

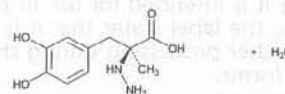
*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 111 mg of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ), to a 50-mL volumetric flask, dissolve in *Diluting solvent* with the aid of sonication, dilute with *Diluting solvent* to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluting solvent* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the Assay under Carbenicillin Indanyl Sodium. Calculate the quantity, in mg, of carbenicillin ( $C_{17}H_{18}N_2O_6S$ ) in the portion of Tablets taken by the formula:

$$0.5CP(r_U/r_S)$$

in which the terms are as defined therein.

## Carbidopa



$C_{10}H_{14}N_2O_4 \cdot H_2O$  244.24

$C_{10}H_{14}N_2O_4$  226.23

Benzenepropanoic acid,  $\alpha$ -hydrazino-3,4-dihydroxy- $\alpha$ -methyl-, monohydrate, (S)-;  
(-)-L- $\alpha$ -Hydrazino-3,4-dihydroxy- $\alpha$ -methylhydrocinnamic acid monohydrate [38821-49-7].

Anhydrous [28860-95-9].

### DEFINITION

Carbidopa contains NLT 98.0% and NMT 102.0% of carbidopa ( $C_{10}H_{14}N_2O_4 \cdot H_2O$ ).

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Mobile phase:** Alcohol and 0.05 M monobasic sodium phosphate, adjusted with phosphoric acid to a pH of 2.7 (5:95)

**System suitability solution:** 0.1 mg/mL of USP Carbidopa RS and 0.1 mg/mL of USP Methyldopa RS in *Mobile phase*

**Standard solution:** 0.5 mg/mL of USP Carbidopa RS in *Mobile phase*. [NOTE—Use gentle heat and ultrasonification, if necessary, to dissolve.]

**Sample solution:** 0.5 mg/mL of Carbidopa in *Mobile phase*



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 280 nm**Column:** 3.9-mm × 30-cm; packing L1**Flow rate:** 1 mL/min**Injection volume:** 20 µL**System suitability****Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for methyl dopa and carbidopa are about 0.8 and 1.0, respectively.]

**Suitability requirements****Resolution:** NLT 0.9 between methyl dopa and carbidopa, *System suitability solution***Relative standard deviation:** NMT 1.5%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*  
Calculate the concentration, in mg/mL, of carbidopa ( $C_{10}H_{14}N_2O_4 \cdot H_2O$ ) in the *Standard solution* ( $C_S$ ):

$$\text{Result} = C_{S2} \times (M_{r1}/M_{r2})$$

 $C_{S2}$  = concentration of USP Carbidopa RS, as determined using the value on the USP Reference Standard label, in the *Standard solution* (mg/mL) $M_{r1}$  = molecular weight of carbidopa monohydrate, 244.24 $M_{r2}$  = molecular weight of anhydrous carbidopa, 226.23Calculate the percentage of carbidopa ( $C_{10}H_{14}N_2O_4 \cdot H_2O$ ) in the portion of Carbidopa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of carbidopa ( $C_{10}H_{14}N_2O_4 \cdot H_2O$ ) in the *Standard solution* (mg/mL) $C_U$  = concentration of Carbidopa in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0%**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**Delete the following:**

- **HEAVY METALS, Method II** (231): NMT 10 ppm • (Official 1-Jan-2018)

- **LIMIT OF METHYLDOPA AND CARBIDOPA RELATED COMPOUND A**

**Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Impurity standard solution:** 2.5 µg/mL of USP Methyl dopa RS and 2.5 µg/mL of USP Carbidopa RS in *Mobile phase*

**Analysis****Samples:** *Sample solution* and *Impurity standard solution*

[NOTE—The relative retention times for methyl dopa, carbidopa, and carbidopa related compound A are about 0.8, 1.0, and 1.8, respectively.]

Calculate the percentage of methyl dopa in the portion of Carbidopa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of methyl dopa from the *Sample solution* $r_S$  = peak response of methyl dopa from the *Impurity standard solution* $C_S$  = concentration of USP Methyl dopa RS in the *Impurity standard solution* (µg/mL) $C_U$  = concentration of the *Sample solution* (µg/mL)

Calculate the percentage of carbidopa related compound A in the portion of Carbidopa taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of carbidopa related compound A from the *Sample solution* $r_S$  = peak response of carbidopa from the *Impurity standard solution* $C_S$  = concentration of USP Carbidopa RS in the *Impurity standard solution* (µg/mL) $C_U$  = concentration of the *Sample solution* (µg/mL)**Acceptance criteria:** NMT 0.5% of methyl dopa and NMT 0.5% of carbidopa related compound A**SPECIFIC TESTS**

- **OPTICAL ROTATION, Specific Rotation** (781S)

**Sample solution:** 10 mg/mL in 0.7 g/mL of aluminum chloride solution (prepared using the hexahydrate form of the aluminum salt) that has been filtered and adjusted with 0.25 N sodium hydroxide to a pH of 1.5

**Acceptance criteria:** −21.0° to −23.5° calculated as the monohydrate

- **LOSS ON DRYING** (731)

**Analysis:** Heat 1 g in a suitable vacuum drying apparatus at 100° and a pressure of NMT 5 mm of mercury to constant weight. Cool, and weigh.

**Acceptance criteria:** 6.9%–7.9%**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

- **USP REFERENCE STANDARDS** (11)

USP Carbidopa RS

USP Methyl dopa RS

**Carbidopa and Levodopa Tablets****DEFINITION**

Carbidopa and Levodopa Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and levodopa ( $C_9H_{11}NO_4$ ).

**IDENTIFICATION**

- **A.**

**Diluent:** 0.05 N hydrochloric acid and methanol (50:50)

**Standard solution A:** 0.1 mg/mL of USP Carbidopa RS in *Diluent*

**Standard solution B:** 0.1 mg/mL of USP Levodopa RS in *Diluent*

**Sample solution:** Nominally 0.1 mg/mL of carbidopa from powdered Tablets in solution, prepared as follows. Transfer a portion of powdered Tablets to a suitable volumetric flask containing 50% of the final volume of 0.05 N hydrochloric acid. Agitate for 20 min. Add methanol to volume, and filter or centrifuge.

**Chromatographic system**(See *Chromatography* (621), *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** 0.25-mm layer of chromatographic silica gel**Application volume:** 20 µL

**Developing solvent system:** Acetone, chloroform, *n*-butanol, glacial acetic acid, and water (27.9: 18.6: 18.6: 18.6: 16.3)



**Spray reagent:** 0.3 g of ninhydrin in 100 mL of *n*-butanol with 3 mL of glacial acetic acid

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop, using the *Developing solvent system*, until the solvent front has moved 15 cm. Air-dry, spray uniformly with 0.5 mL of *Spray reagent*, and heat at 105° for 10 min.

**Acceptance criteria:** The *Sample solution* exhibits two spots (reddish brown for levodopa and yellow-orange for carbidopa) having *R<sub>f</sub>* values that correspond to those exhibited by the *Standard solutions*.

- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Diluent:** 0.24 g/L of sodium 1-decanesulfonate in water

**Mobile phase:** 11.0 g/L of monobasic sodium phosphate in solution, prepared as follows. Transfer a sufficient quantity of monobasic sodium phosphate into a container, and dissolve in water, using 95% of the final volume. Add 0.13% of the final volume of *Diluent*, and adjust with phosphoric acid to a pH of 2.8. Transfer to a suitable volumetric flask, and dilute with water to volume. Pass through a membrane filter.

**Standard solution:** 0.5 mg/mL of USP Levodopa RS and a quantity of USP Carbidopa RS, which is in a ratio with USP Levodopa RS that corresponds to the ratio of carbidopa to levodopa in the Tablets, in solution, prepared as follows. Transfer USP Levodopa RS and USP Carbidopa RS to a suitable volumetric flask, and dissolve in 0.1 N phosphoric acid, using 10% of the final volume. Warm gently to dissolve the Reference Standards, and dilute with water to volume.

**Sample solution:** Nominally 0.5 mg/mL of levodopa from a suitable amount of powdered Tablets in solution, prepared as follows. Transfer a portion of fine powder from NLT 20 Tablets to a suitable volumetric flask. Add 10% of the final volume of 0.1 N phosphoric acid. Dilute with water to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min adjusted, as needed, to obtain retention times for levodopa and carbidopa of 4 min and 11 min, respectively

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 6 between levodopa and carbidopa

**Relative standard deviation:** NMT 2.0% for levodopa; NMT 2.0% for carbidopa

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of carbidopa (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>) and levodopa (C<sub>9</sub>H<sub>11</sub>NO<sub>4</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response of carbidopa or levodopa from the *Sample solution*

*r<sub>S</sub>* = peak response of carbidopa or levodopa from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of carbidopa or levodopa in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of carbidopa; 90.0%–110.0% of the labeled amount of levodopa

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid; 750 mL

**Apparatus 1:** 50 rpm

**Time:** 30 min

**Diluent, Mobile phase, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** (*L<sub>1</sub>*/750) mg/mL of USP Levodopa RS and (*L<sub>2</sub>*/750) mg/mL of USP Carbidopa RS in *Medium*, where *L<sub>1</sub>* and *L<sub>2</sub>* are the label claims of levodopa and carbidopa, respectively, in mg/Tablet

**Sample solution:** A filtered portion of solution under test

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of carbidopa (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>) and levodopa (C<sub>9</sub>H<sub>11</sub>NO<sub>4</sub>) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

*r<sub>U</sub>* = peak response of carbidopa or levodopa from the *Sample solution*

*r<sub>S</sub>* = peak response of carbidopa or levodopa from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

*V* = volume of the *Medium*, 750 mL

*L* = label claim of carbidopa or levodopa (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amounts of carbidopa (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>) and levodopa (C<sub>9</sub>H<sub>11</sub>NO<sub>4</sub>) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

- **USP REFERENCE STANDARDS (11)**

USP Carbidopa RS

USP Levodopa RS

## Carbidopa and Levodopa Extended-Release Tablets

#### DEFINITION

Carbidopa and Levodopa Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of carbidopa (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>) and levodopa (C<sub>9</sub>H<sub>11</sub>NO<sub>4</sub>).

#### IDENTIFICATION

- **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

Protect the volumetric preparations from light.

**Solution A:** 0.24 g/L of sodium 1-decanesulfonate in water

**Solution B:** 11.6 g/L of monobasic sodium phosphate in water

**Mobile phase:** *Solution A*, *Solution B*, and water (0.13:95:4.87), prepared as follows. Add 0.13% of the final volume of *Solution A* to 95% of the final volume of



**Solution B.** Adjust with phosphoric acid to a pH of 2.8. Dilute with water to final volume.

**Standard solution:** 0.1 mg/mL of USP Carbidopa RS and 0.4 mg/mL of USP Levodopa RS in solution, prepared as follows. Transfer accurately weighed portions of the Reference Standards into a suitable volumetric flask, and dissolve in 0.1 N phosphoric acid using 8% of the final volume. Sonication may be used to promote dissolution. Dilute with water to the final volume.

**Sample solution:** Nominally 0.1 mg/mL of carbidopa and 0.4 mg/mL of levodopa from NLT 20 finely powdered Tablets, prepared as follows. Transfer an accurately weighed portion of the powder, equivalent to 1 Tablet weight, into a suitable volumetric flask, and dissolve in 0.1 N phosphoric acid, using 10% of the final volume. Sonicate for 10 min and then stir for 30 min. Dilute with water to volume and stir for another 20 min. Pass the solution through a suitable filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** Standard solution

[NOTE—The relative retention times for levodopa and carbidopa are 1.0 and 2.8, respectively.]

#### Suitability requirements

**Tailing factor:** NMT 1.5 for carbidopa; NMT 1.5 for levodopa

**Resolution:** NLT 6 between levodopa and carbidopa

**Relative standard deviation:** NMT 1.0% for carbidopa; NMT 1.0% for levodopa

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of carbidopa ( $C_{10}H_{14}N_2O_4$ ) or levodopa ( $C_9H_{11}NO_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of carbidopa or levodopa from the Sample solution

$r_s$  = peak response of carbidopa or levodopa from the Standard solution

$C_s$  = concentration of USP Carbidopa RS or USP Levodopa RS in the Standard solution (mg/mL)

$C_u$  = nominal concentration of carbidopa or levodopa in the Sample solution (mg/mL)

**Acceptance criteria:** 90.0%–110.0% each of the labeled amounts of carbidopa and levodopa

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

##### Test 1

**Medium:** 0.1 N hydrochloric acid; 900 mL degassed with helium

**Apparatus 2:** 50 rpm

##### Times

**For Tablets that contain 25 mg of carbidopa and 100 mg of levodopa:** 0.5, 1, and 4 h

**For Tablets that contain 50 mg of carbidopa and 200 mg of levodopa:** 0.5, 1, 2.5, and 4 h

**Solution A:** 0.24 g/L of sodium 1-decanesulfonate in water

**Solution B:** 12.7 g/L of monobasic sodium phosphate in water

**Mobile phase:** Solution A, Solution B, and water (0.13: 95: 4.87), prepared as follows. Add 0.13% of the final volume of Solution A to 95% of the final vol-

ume of Solution B. Adjust with phosphoric acid to a pH of 2.8. Dilute with water to final volume.

**Standard solution:** 0.03 mg/mL of USP Carbidopa RS and 0.1 mg/mL of USP Levodopa RS in Medium. Sonication may be used to aid in dissolution.

#### Sample solution

**For Tablets that contain 25 mg of carbidopa and 100 mg of levodopa:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size and discard the first 1–3 mL.

**For Tablets that contain 50 mg of carbidopa and 200 mg of levodopa:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size, discard the first 1–3 mL, and dilute with Medium (50:50).

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** Standard solution

[NOTE—The relative retention times for levodopa and carbidopa are 0.4 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between levodopa and carbidopa

**Relative standard deviation:** NMT 2.0% for carbidopa and NMT 2.0% for levodopa for six replicate injections

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the concentration ( $C_i$ ) of carbidopa ( $C_{10}H_{14}N_2O_4$ ) or levodopa ( $C_9H_{11}NO_4$ ) in the sample withdrawn from the vessel at each time point  $i$ :

$$\text{Result} = (r_u/r_s) \times C_s \times D$$

$r_u$  = peak response of carbidopa or levodopa from the Sample solution

$r_s$  = peak response of carbidopa or levodopa from the Standard solution

$C_s$  = concentration of USP Carbidopa RS or USP Levodopa RS in the Standard solution (mg/mL)

$D$  = dilution factor for the Sample solution, if needed

Calculate the percentage of the labeled amount of carbidopa ( $C_{10}H_{14}N_2O_4$ ) or levodopa ( $C_9H_{11}NO_4$ ) dissolved at each time point  $i$ :

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_3)] + [C_1 \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_3)]] + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times [V - (3 \times V_3)]] + [(C_3 + C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$C_i$  = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point  $i$  (mg/mL)

$V$  = volume of the Medium, 900 mL

$L$  = label claim of carbidopa or levodopa (mg/ Tablet)

$V_3$  = volume of the Sample solution withdrawn from the Medium (mL)



**Tolerances**

For Tablets that contain 25 mg of carbidopa and 100 mg of levodopa: See Table 1.

**Table 1**

Time Point (i)	Time (h)	Amount of Carbidopa Dissolved	Amount of Levodopa Dissolved
1	0.5	15%–40%	14%–39%
2	1	37%–62%	36%–61%
3	4	NLT 80%	NLT 80%

For Tablets that contain 50 mg of carbidopa and 200 mg of levodopa: See Table 2.

**Table 2**

Time Point (i)	Time (h)	Amount of Carbidopa Dissolved	Amount of Levodopa Dissolved
1	0.5	8%–33%	8%–33%
2	1	26%–51%	26%–51%
3	2.5	62%–87%	64%–89%
4	4	NLT 80%	NLT 80%

The percentages of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and levodopa ( $C_9H_{11}NO_4$ ) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** Simulated gastric fluid TS (prepared without enzymes); 900 mL

**Apparatus 2:** 50 rpm

**Times:** 0.5, 1, 2, and 3 h

**Buffer:** 6.8 g/L of monobasic potassium phosphate and 1.0 g/L of 1-hexanesulfonic acid in water. Adjust with phosphoric acid to a pH of 3.3.

**Mobile phase:** Filtered and degassed mixture of methanol and Buffer (20:80)

**Standard solution:** (L/900) mg/mL each of USP Carbidopa RS and USP Levodopa RS in Medium, where L is the label claim, in mg/Tablet

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** Standard solution

[NOTE—The relative retention times for levodopa and carbidopa are 1.0 and 1.4, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between levodopa and carbidopa

**Column efficiency:** NLT 4000 theoretical plates for both carbidopa and levodopa

**Tailing factor:** NMT 2.0 for both carbidopa and levodopa

**Relative standard deviation:** NMT 1.0% for both carbidopa and levodopa

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the concentration ( $C_i$ ) of carbidopa ( $C_{10}H_{14}N_2O_4$ ) or levodopa ( $C_9H_{11}NO_4$ ) in the sample withdrawn from the vessel at each time point  $i$ :

$$\text{Result} = (r_u/r_s) \times C_s$$

$r_u$  = peak response of carbidopa or levodopa from the Sample solution

$r_s$  = peak response of carbidopa or levodopa from the Standard solution

$C_s$  = concentration of USP Carbidopa RS or USP Levodopa RS in the Standard solution (mg/mL)

Calculate the percentage of the labeled amount of carbidopa ( $C_{10}H_{14}N_2O_4$ ) or levodopa ( $C_9H_{11}NO_4$ ) dissolved at each time point  $i$ :

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_s)] + [C_1 \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_s)]] + [(C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times [V - (3 \times V_s)]] + [(C_3 + C_2 + C_1) \times V_s]\} \times (1/L) \times 100$$

$C_i$  = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point  $i$  (mg/mL)

$V$  = volume of the Medium, 900 mL

$L$  = label claim of carbidopa or levodopa (mg/Tablet)

$V_s$  = volume of the Sample solution withdrawn from the Medium (mL)

**Tolerances:** See Table 3.

**Table 3**

Time Point (i)	Time (h)	Amount Dissolved
1	0.5	20%–35%
2	1	35%–60%
3	2	65%–95%
4	3	NLT 80%

The percentages of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and levodopa ( $C_9H_{11}NO_4$ ) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium, Apparatus 2, Solution A, Solution B, Mobile phase, Standard solution, Chromatographic system, and System suitability:** Proceed as directed in *Test 1*.

**Times:** 0.5, 1, 2.5, and 4 h

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Analysis:** Proceed as directed in *Test 1*.

**Tolerances:** See Table 4.

**Table 4**

Time Point (i)	Time (h)	Amount Dissolved for Tablets That Contain 25 mg of Carbidopa and 100 mg of Levodopa	Amount Dissolved for Tablets That Contain 50 mg of Carbidopa and 200 mg of Levodopa
1	0.5	15%–40%	15%–35%
2	1	25%–65%	25%–65%
3	2.5	NLT 60%	NLT 60%
4	4	NLT 80%	NLT 80%

The percentages of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and levodopa ( $C_9H_{11}NO_4$ ) dissolved at



the times specified conform to *Dissolution* <711>, *Acceptance Table 2*.

**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Times:** 1, 3, and 6 h

**Solution A:** 0.24 g/L of sodium 1-decanesulfonate in water

**Solution B:** 11.6 g/L of monobasic sodium phosphate in water

**Mobile phase:** *Solution A*, *Solution B*, and water (0.13: 95: 4.87), prepared as follows. Add 0.13% of the final volume of *Solution A* to 95% of the final volume of *Solution B*. Adjust with phosphoric acid to a pH of 2.8. Dilute with water to final volume.

**Standard solution:** (L/900) mg/mL each of USP Carbidopa RS and USP Levodopa RS in *Medium*, where L is the label claim, in mg/Tablet

**Sample solution:** Withdraw a 10.0-mL aliquot at each time point and pass a portion of the solution under test through a suitable filter. Replace the 10.0-mL aliquot withdrawn for analysis with a 10.0-mL aliquot of *Medium*.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm × 30-cm; 10-μm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 50 μL

**Run time:** NLT 3 times the retention time of levodopa

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for levodopa and carbidopa are 1.0 and 2.5, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between levodopa and carbidopa

**Tailing factor:** NMT 2.0 for both carbidopa and levodopa

**Relative standard deviation:** NMT 2.0% for both carbidopa and levodopa

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration ( $C_i$ ) of carbidopa ( $C_{10}H_{14}N_2O_4$ ) or levodopa ( $C_9H_{11}NO_4$ ) in the sample withdrawn from the vessel at each time point  $i$ :

$$\text{Result} = (r_u/r_s) \times C_s$$

$r_u$  = peak response of carbidopa or levodopa from the *Sample solution*

$r_s$  = peak response of carbidopa or levodopa from the *Standard solution*

$C_s$  = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of carbidopa ( $C_{10}H_{14}N_2O_4$ ) or levodopa ( $C_9H_{11}NO_4$ ) dissolved at each time point  $i$ :

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_s)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_3 \times V) + (C_2 + C_1) \times V_s] \times (1/L) \times 100$$

$C_i$  = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point  $i$  (mg/mL)

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim of carbidopa or levodopa (mg/Tablet)

$V_s$  = volume of the *Sample solution* withdrawn from the vessel and replaced with *Medium*, 10 mL

**Tolerances:** See *Table 5*.

**Table 5**

Time Point (i)	Time (h)	Amount Dissolved for Tablets That Contain 25 mg of Carbidopa and 100 mg of Levodopa	Amount Dissolved for Tablets That Contain 50 mg of Carbidopa and 200 mg of Levodopa
1	1	35%–70%	25%–60%
2	3	NLT 65%	NLT 65%
3	6	NLT 80%	NLT 80%

The percentages of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and levodopa ( $C_9H_{11}NO_4$ ) dissolved at the times specified conform to *Dissolution* <711>, *Acceptance Table 2*.

**Test 5:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 5*.

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Times:** 0.5, 1, 2.5, and 4 h

**Mobile phase:** 13.6 g/L of monobasic potassium phosphate adjusted with phosphoric acid to a pH of 3.0

**Standard solution:** (L/900) mg/mL each of USP Carbidopa RS and USP Levodopa RS in *Medium*, where L is the label claim, in mg/Tablet. [NOTE—This solution is stable for 1 day if stored at 23°–27°.]

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size, and discard the first 4–5 mL.

[NOTE—This solution is stable for 1 day if stored at 23°–27°.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 282 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

**Run time:** NLT 3 times the retention time of levodopa

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for levodopa and carbidopa are 1.0 and 1.6, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between levodopa and carbidopa

**Tailing factor:** NMT 2.0 for both carbidopa and levodopa

**Relative standard deviation:** NMT 2.0% for both carbidopa and levodopa

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration ( $C_i$ ) of carbidopa ( $C_{10}H_{14}N_2O_4$ ) or levodopa ( $C_9H_{11}NO_4$ ) in the sample withdrawn from the vessel at each time point  $i$ :

$$\text{Result} = (r_u/r_s) \times C_s$$

$r_u$  = peak response of carbidopa or levodopa from the *Sample solution*

$r_s$  = peak response of carbidopa or levodopa from the *Standard solution*

$C_s$  = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)



Calculate the percentage of the labeled amount of carbidopa ( $C_{10}H_{14}N_2O_4$ ) or levodopa ( $C_9H_{11}NO_4$ ) dissolved at each time point  $i$ :

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times (V - V_3)) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_3 \times [V - (2 \times V_3)]) + [(C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

$$\text{Result}_4 = [(C_4 \times [V - (3 \times V_3)]) + [(C_3 + C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

$C_i$  = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point  $i$  (mg/mL)

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim of carbidopa or levodopa (mg/Tablet)

$V_3$  = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See Table 6.

Table 6

Time Point (i)	Time (h)	Amount Dissolved for Tablets That Contain 25 mg of Carbidopa and 100 mg of Levodopa	Amount Dissolved for Tablets That Contain 50 mg of Carbidopa and 200 mg of Levodopa
1	0.5	25%–45%	20%–40%
2	1	40%–65%	30%–60%
3	2.5	NLT 65%	NLT 55%
4	4	NLT 80%	NLT 75%

The percentages of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and levodopa ( $C_9H_{11}NO_4$ ) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

**Test 6:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 6*.

**Medium:** 0.1 N hydrochloric acid; 900 mL degassed under vacuum

**Apparatus 1:** 75 rpm

**Times:** 0.5, 1, 2.5, and 3.5 h

**Solution A:** 0.24 g/L of sodium 1-decanesulfonate in water

**Mobile phase:** To each L of 12.5 g/L of monobasic sodium phosphate dihydrate, add 1.3 mL of *Solution A* and adjust with phosphoric acid to a pH of 2.8.

**Standard solution:** 0.03 mg/mL of USP Carbidopa RS and 0.11 mg/mL of USP Levodopa RS in *Medium*

**Sample solution**

**For Tablets that contain 25 mg of carbidopa and 100 mg of levodopa:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size, discard the first 2 mL, and use the remaining filtrate. Use within 24 h.

**For Tablets that contain 50 mg of carbidopa and 200 mg of levodopa:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size, discard the first 2 mL, and dilute with *Medium* (50:50). Use within 24 h.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

**Run time:** NLT 3 times the retention time of levodopa

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for levodopa and carbidopa are 1.0 and 2.8, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between levodopa and carbidopa

**Tailing factor:** NMT 2.0 for both levodopa and carbidopa

**Relative standard deviation:** NMT 2.0% for both levodopa and carbidopa

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration ( $C_i$ ) of carbidopa

( $C_{10}H_{14}N_2O_4$ ) or levodopa ( $C_9H_{11}NO_4$ ) in the sample withdrawn from the vessel at each time point  $i$ :

$$\text{Result} = (r_u/r_s) \times C_s \times D$$

$r_u$  = peak response of carbidopa or levodopa from the *Sample solution*

$r_s$  = peak response of carbidopa or levodopa from the *Standard solution*

$C_s$  = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

$D$  = dilution factor for the *Sample solution*, if needed

Calculate the percentage of the labeled amount of carbidopa ( $C_{10}H_{14}N_2O_4$ ) or levodopa ( $C_9H_{11}NO_4$ ) dissolved at each time point  $i$ :

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times (V - V_3)) + (C_1 \times V_3)] \times (1/L) \times 100$$

$$\text{Result}_3 = [(C_3 \times [V - (2 \times V_3)]) + [(C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

$$\text{Result}_4 = [(C_4 \times [V - (3 \times V_3)]) + [(C_3 + C_2 + C_1) \times V_3]] \times (1/L) \times 100$$

$C_i$  = concentration of carbidopa or levodopa in the portion of sample withdrawn at time point  $i$  (mg/mL)

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim of carbidopa or levodopa (mg/Tablet)

$V_3$  = volume of the *Sample solution* withdrawn from the *Medium* (mL)

Tolerances: See Table 7.

Table 7

Time Point (i)	Time (h)	Amount Dissolved for Tablets That Contain 25 mg of Carbidopa and 100 mg of Levodopa	Amount Dissolved for Tablets That Contain 50 mg of Carbidopa and 200 mg of Levodopa
1	0.5	15%–40%	10%–30%
2	1	35%–60%	25%–50%
3	2.5	NLT 70%	NLT 65%
4	3.5	NLT 85%	NLT 80%



The percentages of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and levodopa ( $C_9H_9NO_4$ ) dissolved at the times specified conform to *Dissolution* (711), *Acceptance Table 2*.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

Protect all analytical solutions from light and maintain them at 2°–8° until they are injected.

**Buffer:** 6 g/L of anhydrous monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 2.2.

**Mobile phase:** Alcohol and Buffer (5:95)

**System suitability solution:** 1 µg/mL of USP Levodopa Related Compound B RS and 125 µg/mL of USP Carbidopa RS in *Mobile phase*

**Standard solution:** 1.25 µg/mL of USP Carbidopa RS and 5 µg/mL of USP Levodopa RS in *Mobile phase*

**Sensitivity solution:** 0.125 µg/mL of USP Carbidopa RS and 0.5 µg/mL of USP Levodopa RS in *Mobile phase* from the *Standard solution*

**Sample solution:** Nominally 0.125 mg/mL of carbidopa and nominally 0.5 mg/mL of levodopa in *Mobile phase* from NLT 10 finely powdered Tablets, prepared as follows. Transfer an accurately weighed portion of the powder into a suitable volumetric flask, dissolve in *Mobile phase*, and pass through a suitable filter.

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Run time:** 6 times the retention time of carbidopa

**Autosampler temperature:** 6°

**Injection volume:** 20 µL

### System suitability

**Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—For the relative retention times, see *Table 8*.]

### Suitability requirements

**Resolution:** NLT 1.5 between carbidopa and levodopa related compound B, *System suitability solution*

**Relative standard deviation:** NMT 3.0% for both carbidopa and levodopa for five replicate injections, *Standard solution*

**Signal-to-noise ratio:** NLT 10 for carbidopa, *Sensitivity solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of dihydroxybenzaldehyde, dihydroxyphenylacetone, and any unspecified carbidopa degradant based on the label claim of carbidopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of dihydroxybenzaldehyde, dihydroxyphenylacetone, or any unspecified carbidopa degradant from the *Sample solution*

$r_S$  = peak response of carbidopa from the *Standard solution*

$C_S$  = concentration of USP Carbidopa RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carbidopa in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 8*)

Calculate the percentage of levodopa related compound A and any unspecified levodopa degradant

based on the label claim of levodopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of levodopa related compound A or any unspecified levodopa degradant from the *Sample solution*

$r_S$  = peak response of levodopa from the *Standard solution*

$C_S$  = concentration of USP Levodopa RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of levodopa in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 8*)

**Acceptance criteria:** See *Table 8*. For peaks associated with carbidopa, disregard peaks less than 0.05% of carbidopa from the *Sample solution*. For peaks associated with levodopa, disregard peaks less than 0.05% of levodopa from the *Sample solution*.

Table 8

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levodopa related compound A <sup>a,b</sup>	0.9	0.8	0.1
Levodopa	1.0	—	—
Methyldopa <sup>c,d</sup>	1.9	—	—
Levodopa related compound B <sup>a,d</sup>	2.1	—	—
Carbidopa	2.3	—	—
Dihydroxybenzaldehyde <sup>e,f</sup>	5.7	5.9	0.2
Dihydroxyphenylacetone <sup>e,f</sup>	6.3	1.0	1
3-O-Methylcarbidopa <sup>d,g</sup>	6.9	—	—
Any unspecified carbidopa degradant	—	1.0	0.2
Any unspecified levodopa degradant	—	1.0	0.1
Total degradants	—	—	4.0

<sup>a</sup> Individual impurity based on label claim of levodopa.

<sup>b</sup> 3-(3,4,6-Trihydroxyphenyl)alanine.

<sup>c</sup> Individual impurity based on label claim of carbidopa.

<sup>d</sup> This impurity is listed for information only. It is monitored in the drug substance. This impurity is not to be reported and is not to be included in the total degradants.

<sup>e</sup> 3,4-Dihydroxybenzaldehyde.

<sup>f</sup> 3,4-Dihydroxyphenylacetone.

<sup>g</sup> (S)-2-Hydrazinyl-3-(4-hydroxy-3-methoxyphenyl)-2-methylpropanoic acid.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS** (11)
  - USP Carbidopa RS
  - USP Levodopa RS
  - USP Levodopa Related Compound B RS
  - 3-Methoxytyrosine.
  - $C_{10}H_{13}NO_4$  211.21



## Carbidopa and Levodopa Orally Disintegrating Tablets

### DEFINITION

Carbidopa and Levodopa Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and levodopa ( $C_9H_{11}NO_4$ ).

### IDENTIFICATION

- A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

Protect the volumetric solutions from light.

**Buffer:** 6.6 g/L of monobasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 2.2

**Mobile phase:** Alcohol and Buffer (5:95)

**Standard solution:** 0.025 mg/mL of USP Carbidopa RS and 0.25 mg/mL of USP Levodopa RS in *Mobile phase*

**Sample stock solution:** Transfer NLT 10 Tablets to a 1-L volumetric flask. Add 750 mL of *Mobile phase*, sonicate for 20 min, and then stir for 20 min. Dilute with *Mobile phase* to volume.

**Sample solution:** Dilute the *Sample stock solution* with *Mobile phase* to obtain a nominal concentration of carbidopa of between 0.025 and 0.07 mg/mL and a nominal concentration of levodopa of 0.25 mg/mL.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Autosampler temperature:** 6°

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for levodopa and carbidopa are 0.42 and 1.0, respectively.]

#### Suitability requirements

**Tailing factor:** NMT 2.4 for both the levodopa and carbidopa peaks

**Relative standard deviation:** NMT 2.0% for both carbidopa and levodopa

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and levodopa ( $C_9H_{11}NO_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of carbidopa or levodopa from the *Sample solution*

$r_S$  = peak response of carbidopa or levodopa from the *Standard solution*

$C_S$  = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carbidopa or levodopa in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% each of the labeled amounts of carbidopa and levodopa

### PERFORMANCE TESTS

- DISINTEGRATION (701):** NMT 60 s

- DISSOLUTION (711)**

**Medium:** 0.1 N hydrochloric acid; 750 mL

**Apparatus 2:** 50 rpm

**Time:** 10 min

**Solution A:** 0.24 g/L of sodium 1-decanesulfonate in water

**Mobile phase:** Dissolve 11.0 g of monobasic sodium phosphate monohydrate in 1 L of water. Add 1.3 mL of *Solution A*, and adjust with phosphoric acid to a pH of 2.8.

**Standard solution:** (1/800) mg/mL each of USP Carbidopa RS and USP Levodopa RS in *Medium*, where *L* is the label claim in mg/Tablet of carbidopa or levodopa

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size, and discard the first 3 mL.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  15.0-cm; 5- $\mu$ m packing L1

**Autosampler temperature:** 4°

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for levodopa and carbidopa are 0.4 and 1.0, respectively.]

#### Suitability requirements

**Tailing factor:** NMT 2.0 for both levodopa and carbidopa

**Relative standard deviation:** NMT 2.0% for both levodopa and carbidopa

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of carbidopa ( $C_{10}H_{14}N_2O_4$ ) and levodopa ( $C_9H_{11}NO_4$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response of carbidopa or levodopa from the *Sample solution*

$r_S$  = peak response of carbidopa or levodopa from the *Standard solution*

$C_S$  = concentration of USP Carbidopa RS or USP Levodopa RS in the *Standard solution* (mg/mL)

$V$  = volume of the *Medium*, 750 mL

$L$  = label claim of carbidopa or levodopa (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of carbidopa ( $C_{10}H_{14}N_2O_4$ ) is dissolved, and NLT 75% (Q) of the labeled amount of levodopa ( $C_9H_{11}NO_4$ ) is dissolved.

- UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

### IMPURITIES

#### ORGANIC IMPURITIES

Protect all analytical solutions from light, and maintain them at 2°–8° until they are injected.

**Diluent:** Methanol and 0.1 N hydrochloric acid (30:70)

**Mobile phase:** 13.8 g/L of monobasic sodium phosphate monohydrate in water, adjusted with phosphoric acid to a pH of 2.7

**System suitability solution:** 0.025 mg/mL each of USP Carbidopa RS, USP Levodopa RS, USP Levodopa Related Compound A RS, USP Levodopa Related Compound B RS, and USP Methylidopa RS in *Diluent*

**Standard solution:** 0.025 mg/mL of USP Levodopa RS in *Diluent*

**Sample solution:** Transfer a weighed quantity of powder equivalent to 250 mg of levodopa from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add 80 mL of *Diluent*, sonicate for 10 min, and then stir for 30 min. Dilute with *Diluent* to volume. Centrifuge, and inject the supernatant within 2 h.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Autosampler temperature: 4°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

Run time: 6 times the retention time of carbidopa

**System suitability****Samples:** *System suitability solution* and *Standard solution*[NOTE—For the relative retention times, see *Table 1*. If peak fronting for levodopa related compound A is observed, lowering the column temperature to 15° is recommended to eliminate this problem.]**Suitability requirements****Resolution:** NLT 1.5 between levodopa related compound A and levodopa, NLT 2.0 between carbidopa and levodopa related compound B, and NLT 1.5 between methyldopa and carbidopa; *System suitability solution***Relative standard deviation:** NMT 5.0% for levodopa, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of all impurities and any unspecified degradation product other than methyldopa and 3,4-dihydroxyphenylacetone, based on the label claim of levodopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of levodopa related compound A or any unspecified degradation product from the *Sample solution* $r_S$  = peak response of levodopa from the *Standard solution* $C_S$  = concentration of USP Levodopa RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of levodopa in the *Sample solution* (mg/mL) $F$  = relative response factor (see *Table 1*)

Calculate the percentage of methyldopa and 3,4-dihydroxyphenylacetone based on the label claim of carbidopa in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of methyldopa or 3,4-dihydroxyphenylacetone from the *Sample solution* $r_S$  = peak response of levodopa from the *Standard solution* $C_S$  = concentration of USP Levodopa RS in the *Standard solution* $C_U$  = nominal concentration of carbidopa in the *Sample solution* $F$  = relative response factor (see *Table 1*)**Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Levodopa related compound A <sup>a</sup>	0.45	0.80	0.2
Levodopa	0.52	—	—
Methyldopa <sup>b</sup>	0.84	1.0	0.5
Carbidopa	1.0	—	—
Levodopa related compound B <sup>c</sup>	1.2	—	—
3-O-methyl carbidopa <sup>c</sup>	3.1	—	—
3,4-Dihydroxyphenylacetone <sup>b,d</sup>	3.9	1.0	1.0
Any individual unspecified degradation product <sup>a</sup>	—	1.0	0.2
Total impurities <sup>e</sup>	—	—	1.0

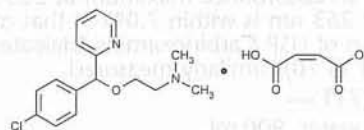
<sup>a</sup> Individual impurity based on the label claim of levodopa.<sup>b</sup> Individual impurity based on the label claim of carbidopa.<sup>c</sup> Process-related impurities, included for identification only; not to be included in *Total impurities*.<sup>d</sup> (S)-2-Hydrazinyl-3-(4-hydroxy-3-methoxyphenyl)-2-methylpropanoic acid.<sup>e</sup> Excluding all process impurities and 3,4-dihydroxyphenylacetone.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers, and store at controlled room temperature.• **USP REFERENCE STANDARDS (11)**

USP Carbidopa RS

USP Levodopa RS

USP Levodopa Related Compound A RS  
3-(3,4,6-Trihydroxyphenyl)alanine.C<sub>9</sub>H<sub>11</sub>NO<sub>5</sub> 213.19USP Levodopa Related Compound B RS  
3-Methoxytyrosine.C<sub>10</sub>H<sub>13</sub>NO<sub>4</sub> 211.21

USP Methyldopa RS

**Carbinoxamine Maleate**C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub>O · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub> 406.86

Ethanamine, 2-[(4-chlorophenyl)-2-pyridinylmethoxy]-N,N-dimethyl-, (Z)-2-butenedioate (1:1).

2-[p-Chloro-α-[2-(dimethylamino)ethoxy]benzyl]pyridine maleate (1:1) [3505-38-2].

» Carbinoxamine Maleate, dried at 105° for 2 hours, contains not less than 98.0 percent and not more than 102.0 percent of C<sub>16</sub>H<sub>19</sub>ClN<sub>2</sub>O · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>.**Packaging and storage**—Preserve in tight, light-resistant containers.**USP Reference standards (11)**—

USP Carbinoxamine Maleate RS



**Identification—****A:** Infrared Absorption (197M).**B:** Ultraviolet Absorption (197U)—

Solution: 50 µg per mL.

Medium: methanol.

Absorptivities at 260 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 116° and 121°, determined after drying.**pH** (791): between 4.6 and 5.1, in a solution (1 in 100).**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.**Residue on ignition** (281): not more than 0.1%.**Ordinary impurities** (466)—

Test solution: chloroform.

Standard solution: chloroform.

Eluant: a mixture of cyclohexane, chloroform, and diethylamine (75:15:10).

Visualization: 1.

**Assay**—Dissolve about 400 mg of Carbinoxamine Maleate, previously dried and accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 20.34 mg of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$ .

## Carbinoxamine Maleate Tablets

» Carbinoxamine Maleate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Carbinoxamine Maleate RS

**Identification**—A solution of carbinoxamine maleate (1 in 50,000) in dilute sulfuric acid (1 in 70) prepared from the Tablets as directed under *Salts of Organic Nitrogenous Bases* (501) exhibits an absorbance maximum at  $263 \pm 2$  nm. The absorptivity at 263 nm is within 7.0% of that of a 1 in 50,000 solution of USP Carbinoxamine Maleate RS in dilute sulfuric acid (1 in 70), similarly measured.

**Dissolution** (711)—

Medium: water; 900 mL.

Apparatus 2: 50 rpm.

Time: 45 minutes.

**Procedure**—Determine the amount of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 260 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Carbinoxamine Maleate RS in the same medium.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Place 1 Tablet in a 100-mL volumetric flask, add 10.0 mL of water, and shake by mechanical means for 15 minutes. Dilute with methanol

to volume, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate quantitatively and stepwise, if necessary, with a mixture of methanol and water (9:1) to obtain a solution containing about 40 µg of carbinoxamine maleate per mL. Concomitantly determine the absorbances of this solution and of a Standard solution of USP Carbinoxamine Maleate RS, in the same medium having a known concentration of about 40 µg per mL, in 1-cm cells, at the wavelength of maximum absorbance at about 260 nm, with a suitable spectrophotometer, using a mixture of methanol and water (9:1) as the blank. Calculate the quantity, in mg, of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$  in the Tablet taken by the formula:

$$(TC/D)(A_U/A_S)$$

in which *T* is the labeled quantity, in mg, of carbinoxamine maleate in the Tablet; *C* is the concentration, in µg per mL, of USP Carbinoxamine Maleate RS in the Standard solution; *D* is the concentration, in µg per mL, of carbinoxamine maleate in the solution from the Tablet, based upon the labeled quantity per Tablet and the extent of dilution; and *A<sub>U</sub>* and *A<sub>S</sub>* are the absorbances of the solution from the Tablet and the Standard solution, respectively.

**Assay**—Weigh and finely powder not less than 30 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of carbinoxamine maleate, to a separator, add 35 mL of water and 3 g of sodium bicarbonate, and mix. Extract with five 20-mL portions of chloroform, filtering the extracts through a pledget of cotton. Evaporate the combined chloroform extracts on a steam bath just to dryness, dissolve the residue in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.05 N perchloric acid VS to a blue-green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.05 N perchloric acid is equivalent to 10.17 mg of  $C_{16}H_{19}ClN_2O \cdot C_4H_4O_4$ .

## Carbol-Fuchsin Topical Solution

» Prepare Carbol-Fuchsin Topical Solution as follows:

Basic Fuchsin .....	3 g
Phenol .....	45 g
Resorcinol .....	100 g
Acetone .....	50 mL
Alcohol .....	100 mL
Purified Water, a sufficient quantity, to make .....	1000 mL

Dissolve the Basic Fuchsin in a mixture of the Acetone and Alcohol, and add to this solution the Phenol and Resorcinol previously dissolved in 725 mL of Purified Water. Then add sufficient Purified Water to make the product measure 1000 mL, and mix.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Specific gravity** (841): not less than 0.990 and not more than 1.050.

**Alcohol Determination** (611): between 7.0% and 10.0% of  $C_2H_5OH$ .



## Carbon Dioxide

CO<sub>2</sub> 44.01  
Carbon dioxide [124-38-9].

### DEFINITION

Carbon Dioxide contains NLT 99.0%, by volume, of carbon dioxide (CO<sub>2</sub>).

[NOTE—The following tests are designed to reflect the quality of Carbon Dioxide in both its vapor and liquid phases, which are present in previously unopened cylinders. Reduce the container pressure by means of a regulator. Withdraw the specimens for the tests with the least possible release of Carbon Dioxide consistent with proper purging of the sampling apparatus. Measure the gases with a gas volume meter downstream from the detector tubes to minimize contamination or change of the specimens.]

### IDENTIFICATION

#### • A.

**Sample:** 100 ± 5 mL, released from the vapor phase of the contents of the container

**Analysis:** Pass the *Sample* through a carbon dioxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** The indicator change extends throughout the entire indicating range of the tube.

### ASSAY

#### • PROCEDURE

[NOTE—Sampling for this Assay may be done from the vapor phase for convenience, but this method results in more residual volume. If the specification of 1 mL is exceeded from the vapor phase, a liquid specimen may be taken.]

**Sample:** 100.0 mL of specimen taken from the liquid phase, as directed in the test for *Nitrogen Dioxide*

**Analysis:** Assemble a 100-mL gas buret, provided with a leveling bulb and two-way stopcock, and a gas absorption pipet of suitable capacity by connecting the pipet to one of the buret outlets. Fill the buret with slightly acidified water (turned pink with methyl orange), and fill the pipet with potassium hydroxide solution (1 in 2). By manipulation of the leveling bulb and leveling water, draw the potassium hydroxide solution to fill the pipet and capillary connection up to the stopcock. Fill the buret with the leveling water, and draw it through the other stopcock opening in such a manner that all gas bubbles are eliminated from the system. Draw the *Sample* into the buret. By raising the leveling bottle, force the measured specimen into the pipet. The absorption may be facilitated by rocking the pipet or by flowing the specimen between pipet and buret. Draw any residual gas into the buret, and measure its volume.

**Acceptance criteria:** NMT 1.0 mL of gas remains (NLT 99.0%, by volume, of CO<sub>2</sub>).

### IMPURITIES

#### • NITROGEN DIOXIDE

**Sample:** 550 ± 50 mL, obtained as directed in the Analysis

**Analysis:** Arrange the container so that when its valve is opened, a portion of the liquid phase of the contents is released through a piece of tubing of sufficient length to allow all of the liquid to vaporize during passage through it, and to prevent frost from reaching the inlet of the detector tube. Release into the tubing a flow of liquid sufficient to provide 550 mL of the vaporized specimen plus any excess necessary to ensure adequate flushing of air from the system. Pass 550 ± 50 mL of this gas through a nitric oxide–nitrogen dioxide detec-

tor tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 2.5 ppm

#### • LIMIT OF AMMONIA

**Sample:** 1050 ± 50 mL of the gas obtained as directed in the test for *Nitrogen Dioxide*

**Analysis:** Proceed with Carbon Dioxide as directed in the test for *Nitrogen Dioxide*, except pass 1050 ± 50 mL of this gas through an ammonia detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 0.0025%

#### • LIMIT OF HYDROGEN SULFIDE

**Sample:** 1050 ± 50 mL, released from the vapor phase

**Analysis:** Pass 1050 ± 50 mL, released from the vapor phase, through a hydrogen sulfide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 1 ppm

#### • LIMIT OF NITRIC OXIDE

**Sample:** 550 ± 50 mL, released from the vapor phase

**Analysis:** Pass 550 ± 50 mL, released from the vapor phase, through a nitric oxide–nitrogen dioxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 2.5 ppm

#### • CARBON MONOXIDE

**Sample:** 1050 ± 50 mL, released from the vapor phase of the contents of the container

**Analysis:** Pass 1050 ± 50 mL, released from the vapor phase of the contents of the container, through a carbon monoxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 0.001%

#### • SULFUR DIOXIDE

**Sample:** 1050 ± 50 mL, obtained as directed in the test for *Nitrogen Dioxide*

**Analysis:** Proceed with Carbon Dioxide as directed in the test for *Nitrogen Dioxide*, except to pass 1050 ± 50 mL through a sulfur dioxide detector tube (see *Reagents, Indicators, and Solutions*) at the rate specified for the tube.

**Acceptance criteria:** NMT 5 ppm

### SPECIFIC TESTS

#### • WATER DETERMINATION

**Analysis:** Flush the regulator that has been flushed with 5 L or more of the gas specimen. Pass 50 ± 5 L, released from the vapor phase, through a water vapor detector tube connected to the regulator with a minimum length of metal or polyethylene tubing. Measure the gas passing through the detector tube with a gas flowmeter set at a flow rate of 2 L/min.

**Acceptance criteria:** NMT 150 mg/m<sup>3</sup>

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in cylinders.

## Urea C 13



Urea [<sup>13</sup>C] 61.05  
[58069-82-2].

### DEFINITION

Urea C 13 contains NLT 98.0% and NMT 102.0% of urea C 13 (<sup>13</sup>CH<sub>4</sub>N<sub>2</sub>O).



**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention times of the major peaks corresponding to the mass-to-charge ( $m/z$ ) ratios of 61 and 63 in the *Sample solution* correspond to those of the *Standard solution*, as obtained in the test for *Isotopic Purity*.

**ASSAY**• **PROCEDURE**

Mobile phase: Acetonitrile, methanol, and water (89:10:1)

System suitability solution: 2.5 mg/mL of urea and 0.003 mg/mL of biuret in *Mobile phase*

Standard solution: 2 mg/mL of USP Urea C 13 RS in *Mobile phase*

Sample solution: 2 mg/mL of Urea C 13 in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L8

Flow rate: 0.8 mL/min

Injection volume: 20  $\mu$ L

**System suitability**

Samples: *System suitability solution* and *Standard solution*

**Suitability requirements**

Resolution: NLT 1.5 between urea and biuret, *System suitability solution*

Relative standard deviation: NMT 1%, *Standard solution*

**Analysis**

Samples: *Standard solution* and *Sample solution*

Measure the areas for the major peaks.

Calculate the percentage of urea C 13 ( $^{13}\text{CH}_4\text{N}_2\text{O}$ ) in the portion of Urea C 13 taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Urea C 13 RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Urea C 13 in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 0.1%

**Delete the following:**

- **HEAVY METALS (231)**

Analysis: Dissolve 1.0 g in 20 mL of water, and add 5 mL of 0.1 N hydrochloric acid.

Acceptance criteria: NMT 20 ppm • (Official 1-Jan-2018)

- **LIMIT OF BIURET**

Standard solution: 0.033 mg/mL of biuret in water

Sample solution: 33.3 mg/mL of Urea C 13 in water

Analysis: Treat 3 mL of the *Sample solution* and 3 mL of the *Standard solution* separately as follows. To each solution add 3 mL of sodium hydroxide solution (10 in 100) and 3 drops of copper sulfate solution (0.5 in 100), and allow to stand for 5 min.

Acceptance criteria: NMT 0.1%; any reddish-violet color in the *Sample solution* is not more intense than that in the *Standard solution*.

- **ISOTOPIC PURITY**

Standard solution: 0.4 mg/mL of USP Urea C 13 RS in methanol

Sample solution: 0.4 mg/mL of Urea C 13 in methanol

**Chromatographic system**

(See *Chromatography* (621) and *Mass Spectrometry* (736).)

Mode: GC

Detector: Mass spectrometer, electron energy 70 eV

Column: 0.25-mm  $\times$  15-m capillary; coated with a 0.1- $\mu$ m film of phase G25 or G35

**Temperatures**

Injection port: 250°

Detector: 200°

Transfer line: 265°

Column: See *Table 1*.

**Table 1**

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
80	15	250	0

Carrier gas: Helium

Flow rate: 1 mL/min

Injection volume: 1  $\mu$ L

Split ratio: 1:25

**Analysis**

Samples: *Sample solution* and *Standard solution*

[NOTE—The *Standard solution* chromatogram is also intended for use in *Identification* test B.]

Record the total ion chromatogram, and combine all of the mass spectra scans across the entire major peak.

Record the peak intensities at mass-to-charge ( $m/z$ ) ratios of 60, 61, 62, and 63.

Calculate the percentage of carbon that is C 13 in the portion of Urea C 13 taken:

$$\text{Result} = [(I_{61} + I_{63})/(I_{60} + I_{61} + I_{63})] \times 100$$

Calculate the percentage of oxygen that is O 18 in the portion of Urea C 13 taken:

$$\text{Result} = [(I_{62} + I_{63})/(I_{60} + I_{61} + I_{62} + I_{63})] \times 100$$

$I_{60}$  = relative peak intensity at an  $m/z$  ratio of 60 in the *Sample solution*

$I_{61}$  = relative peak intensity at an  $m/z$  ratio of 61 in the *Sample solution*

$I_{62}$  = relative peak intensity at an  $m/z$  ratio of 62 in the *Sample solution*

$I_{63}$  = relative peak intensity at an  $m/z$  ratio of 63 in the *Sample solution*

**Acceptance criteria**

C 13: NLT 99%

O 18: NMT 15%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers at room temperature.

- **USP REFERENCE STANDARDS (11)**

USP Urea C 13 RS

**Urea C 13 for Oral Solution****DEFINITION**

Urea C 13 for Oral Solution is a dry powder prepared from Urea C 13. It contains NLT 90.0% and NMT 110.0% of the labeled amount of urea C 13 ( $^{13}\text{CH}_4\text{N}_2\text{O}$ ). It contains no preservatives.

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.



**ASSAY****• PROCEDURE**

**Mobile phase:** Acetonitrile, methanol, and water (89:10:1)

**System suitability solution:** 2.5 mg/mL of urea and 0.003 mg/mL of biuret in *Mobile phase*

**Standard solution:** 2 mg/mL of USP Urea C 13 RS in *Mobile phase*

**Sample solution:** 2 mg/mL of urea C 13 from a portion of Urea C 13 for Oral Solution in *Mobile phase*

**Chromatographic system**

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L8

**Flow rate:** 0.8 mL/min

**Injection volume:** 20 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between urea and biuret, *System suitability solution*

**Relative standard deviation:** NMT 1%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Measure the areas for the major peaks.

Calculate the percentage of the labeled amount of urea C 13 ( $^{13}\text{CH}_4\text{N}_2\text{O}$ ) in the portion of Urea C 13 for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Urea C 13 RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of urea C 13 in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

**SPECIFIC TESTS**

- MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62)**

**Acceptance criteria**

Total aerobic microbial count: NMT  $10^3$  cfu/g

Yeast count: NMT  $10^2$  cfu/g

*Salmonella* species and *Escherichia coli*: Absent

- COMPLETENESS OF SOLUTION (641)**

**Sample solution:** Nominally 100 mg/mL of urea C 13 from a portion of Urea C 13 for Oral Solution in carbon dioxide-free water

**Acceptance criteria:** Meets the requirements

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in sterile, well-closed containers. Store at 15°–30°.
- LABELING:** Label it to indicate that the solution is to be discarded if particulate matter is visible after reconstitution. [NOTE—It is to be reconstituted with sterile purified water.]

**• USP REFERENCE STANDARDS (11)**

USP Urea C 13 RS

**Urea C 14 Capsules**

» Urea C 14 Capsules contain  $^{14}\text{CH}_4\text{N}_2\text{O}$  in which a portion of the molecules are labeled with radio-active  $^{14}\text{C}$  to provide 0.04 MBq (or 1 μCi) of radioactivity per capsule. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{14}\text{C}$  expressed as MBq (or μCi).

**Packaging and storage—**Preserve in tight containers, and store at controlled room temperature.

**Expiration date—**The expiration date is not later than two years from the date of manufacture.

**Labeling—**Label it to include the following: the amount of  $^{14}\text{C}$ , expressed in MBq (or μCi) per Capsule at the time of calibration; the expiration date; the total radioactivity per container; and the statement, "Caution—Radioactive material."

**Radionuclide identification (821)—**A solution of 1 or more Capsules in 1 N hydrochloric acid when tested using a liquid scintillation counter produces beta emission having a 49 keV mean and a 156 keV max.

**Dissolution (711)—**

**Medium:** simulated gastric fluid TS; 500 mL.

**Apparatus 1:** 50 rpm.

**Time:** 10 minutes.

**Procedure—**Determine the background levels of  $^{14}\text{C}$  with a 1-mL portion of the solution under test using a liquid scintillation counter.

**Tolerances:** not less than 80% (Q) of the labeled amount of  $^{14}\text{C}$  is dissolved in 10 minutes.

**Uniformity of dosage units (905):** meet the requirements.

**Radionuclidic purity (821)—**Determine the radionuclidic purity of a solution of 1 or more Capsules in water using a liquid scintillation counter: not less than 99.9% of the radioactivity is present as C 14.

**Radiochemical purity—**

**Adsorbent:** 0.25-mm layer of chromatographic cellulose.

**Test solution—**Open 2 Capsules and place them in a suitable container, add 8 mL of methanol, and mix.

**Reference solution:** 40 mg of urea per mL, in water.

**Application volume:** 20 μL of the *Test solution* and 4 μL of the *Reference solution*.

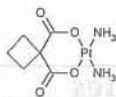
**Developing solvent system:** *n*-butanol saturated with water.

**Procedure—**Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). Locate the spots on the plate by spraying with Ehrlich's reagent. Determine the radioactivity distribution with a suitable radiation detector (see *Radioactivity* (821)), and obtain the  $R_f$  value; the  $R_f$  value of the principal spot from the *Test solution* corresponds to that obtained from the *Reference solution*, and the radioactivity of the  $^{14}\text{C}$  band is not less than 90% of the total radioactivity.

**Assay for radioactivity (821)—**Prepare a solution of 1 or more Capsules in 1 N hydrochloric acid. Using a liquid scintillation counter, determine the radioactivity, in MBq (or mCi) per mL by use of a calibrated system.



## Carboplatin



$C_6H_{12}N_2O_4Pt$  371.25  
Platinum, diammine[1,1-cyclobutanedicarboxylato(2-)- $O,O'$ ], (SP-4-2);  
*cis*-Diammine(1,1-cyclobutanedicarboxylato)platinum  
[41575-94-4].

### DEFINITION

#### Change to read:

Carboplatin contains NLT 98.0% and NMT 102.0% of carboplatin ( $C_6H_{12}N_2O_4Pt$ ), calculated on the  $\Delta$ dried $\Delta_{USP40}$  basis.

[CAUTION—Great care should be taken in handling Carboplatin because it is a suspected carcinogen.]

### IDENTIFICATION

#### Change to read:

- **A.  $\Delta$ INFRARED ABSORPTION (197)**  
[NOTE—Methods described in (197K) or (197A) may be used.] $\Delta_{USP40}$

#### Add the following:

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. $\Delta_{USP40}$

### ASSAY

#### Change to read:

#### • PROCEDURE

**Mobile phase:** Acetonitrile and water (87:13)

**Standard solution:** 1 mg/mL of USP Carboplatin RS in water. Use it within 2 h.

**Sample solution:** 1 mg/mL of Carboplatin in water. Use it within 2 h.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:**  $\Delta$ 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L8 $\Delta_{USP40}$

**Flow rate:** 2.0 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

$\Delta_{USP40}$

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 1.2%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carboplatin ( $C_6H_{12}N_2O_4Pt$ ) in the portion of Carboplatin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*  
 $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Carboplatin RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the  $\Delta$ dried $\Delta_{USP40}$  basis

### OTHER COMPONENTS

#### Change to read:

#### • PLATINUM CONTENT

**Sample:** 0.2 g of Carboplatin, from *Loss on Drying*

**Analysis:** Ignite the *Sample* to constant weight at  $800 \pm 50^\circ$ , and weigh the residue. The residue is platinum.

Calculate the platinum content in the portion of Carboplatin taken:

$$\text{Result} = (W_u/W_s) \times 100$$

$W_u$  = weight of platinum

$W_s$  = weight of *Sample*

Acceptance criteria: 52.0%–53.0% on the dried basis $\Delta_{USP40}$

### IMPURITIES

#### Change to read:

#### • LIMIT OF 1,1-CYCLOBUTANEDICARBOXYLIC ACID

**Solution A:** Dissolve 8.5 g of tetrabutylammonium hydrogen sulfate in 80 mL of water. Add 3.4 mL of phosphoric acid, and adjust with 10 N sodium hydroxide to a pH of 7.55.

**Mobile phase:** Acetonitrile, *Solution A*, and water (100:20:880)

**Standard solution:** 5  $\mu$ g/mL of 1,1-cyclobutanedicarboxylic acid in *Mobile phase*

**System suitability solution:** 2.5  $\mu$ g/mL of 1,1-cyclobutanedicarboxylic acid and 0.5 mg/mL of Carboplatin in *Mobile phase* prepared as follows. Mix 1.0 mL of *Standard solution* with 1.0 mL of *Standard solution* in the *Assay*.

**Sample solution:** 1 mg/mL of Carboplatin in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.0-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 100  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for carboplatin and 1,1-cyclobutanedicarboxylic acid are 0.65 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between the carboplatin and 1,1-cyclobutanedicarboxylic acid peaks

$\Delta_{USP40}$

**Relative standard deviation:** NMT 10%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 1,1-cyclobutanedicarboxylic acid in the portion of Carboplatin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of 1,1-cyclobutanedicarboxylic acid from the *Sample solution*

$r_s$  = peak response of 1,1-cyclobutanedicarboxylic acid from the *Standard solution*

$C_s$  = concentration of 1,1-cyclobutanedicarboxylic acid in the *Standard solution* (mg/mL)



$C_U$  = concentration of Carboplatin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

#### Change to read:

#### • ORGANIC IMPURITIES

▲ Mobile phase, Standard solution, Sample solution, and System suitability: Proceed as directed in the Assay.

Diluted standard solution: 2.5 µg/mL of USP

Carboplatin RS in water, from the *Standard solution*

Chromatographic system: Proceed as directed in the Assay, and the run time is at least 2.5 times the retention time of the carboplatin peak.

#### Analysis

Samples: *Sample solution* and *Diluted standard solution*  
Calculate the percentage of each impurity in the portion of Carboplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of carboplatin from the *Diluted standard solution*

$C_S$  = concentration of USP Carboplatin RS in the *Diluted standard solution* (mg/mL)

$C_U$  = concentration of Carboplatin in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cisplatin*	0.3	0.25
Carboplatin	1.0	—
Any individual unspecified impurity	—	0.25
Total impurities	—	0.5

\* *cis*-Diamminedichloroplatinum(II).

▲ USP40

#### SPECIFIC TESTS

• CRYSTALLINITY (695): Meets the requirements

• PH (791)

Sample solution: 10 mg/mL in water

Acceptance criteria: 5.0–7.0

#### Delete the following:

▲ WATER DETERMINATION, Method I (921): NMT 0.5%, using anhydrous formamide as the solvent.▲ USP40

#### Add the following:

▲ LOSS ON DRYING (731)

Sample: 1 g

Analysis: Dry the *Sample* at 105° to constant weight.

Acceptance criteria: NMT 0.5%▲ USP40

• TRANSMITTANCE

Sample solution: 10 mg/mL of Carboplatin in water

Analysis: Determine the percent transmittance in 1-cm cells at a wavelength of 440 nm, using water as the blank.

Acceptance criteria: NLT 97%

#### • WATER-INSOLUBLE MATTER

Sample: 1 g

Analysis: Transfer the *Sample* to a 150-mL beaker. Add 100 mL of water, and dissolve by stirring with a stirring bar for 30 min. With the aid of suction, pass through a tared filtering crucible. Rinse the beaker with water, and transfer the rinsings to the crucible. Dry the crucible at 130 ± 10° to constant weight.

Acceptance criteria: NMT 0.5%

#### ADDITIONAL REQUIREMENTS

#### Change to read:

• PACKAGING AND STORAGE: Preserve in tight containers, protected from light. ▲ Store at room temperature.▲ USP40

• USP REFERENCE STANDARDS (11)

USP Carboplatin RS

## Carboplatin for Injection

### DEFINITION

Carboplatin for Injection is a sterile, lyophilized mixture of Carboplatin and Mannitol. It contains NLT 90.0% and NMT 110.0% of the labeled amount of carboplatin ( $C_6H_{12}N_2O_4Pt$ ).

[CAUTION—Great care should be taken in handling Carboplatin because it is a suspected carcinogen.]

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHY

Standard solution: 10 mg/mL of USP Carboplatin RS in water

Sample solution: Nominally equivalent to 10 mg/mL of carboplatin in water from the contents of one container

#### Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 10 µL

Developing solvent system: Acetone and water (80:20)

Spray reagent: Add 5.6 g of stannous chloride to 10 mL of hydrochloric acid, and stir for 5 min. [NOTE—It is not necessary that all of the solids dissolve.] Add 90 mL of water and 1 g of potassium iodide, and stir. Prepare this solution fresh daily.

#### Analysis

Samples: *Standard solution* and *Sample solution*

Place the plate in a chromatographic chamber lined with filter paper and equilibrated for 2 h in *Developing solvent system*. Develop the chromatogram until the solvent front has moved 10 cm from the origin. Remove the plate from the chamber, and air-dry at room temperature for 2 h. Spray with the *Spray reagent*, and heat at 110° for 10 min.

Acceptance criteria: The principal spot of the *Sample solution* corresponds in appearance and  $R_f$  value to that of the *Standard solution*.

#### Add the following:

▲ B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.▲ USP40



## ASSAY

## Change to read:

## • PROCEDURE

**Mobile phase:** Acetonitrile and water (87:13)

**Standard solution:** 1 mg/mL of USP Carboplatin RS. Use this solution within 2 h.

**Sample solution:** Nominally equivalent to 1 mg/mL of carboplatin from the contents of one container diluted with water. Complete chromatographic analysis of this solution within 2 h.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:**  $\Delta$ 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L8 $\Delta$ USP40

**Flow rate:** 2.0 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

$\Delta$ USP40

**Tailing factor:** NMT 2.5

**Relative standard deviation:** NMT 1.2%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carboplatin ( $C_6H_{12}N_2O_4Pt$ ) in the portion of Carboplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Carboplatin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carboplatin in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

## PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

## IMPURITIES

## Change to read:

• **LIMIT OF 1,1-CYCLOBUTANEDICARBOXYLIC ACID**

**Solution A:** Dissolve 8.5 g of tetrabutylammonium hydrogen sulfate in 80 mL of water. Add 3.4 mL of phosphoric acid, and adjust with 10 N sodium hydroxide to a pH of 7.55.

**Mobile phase:** Acetonitrile, *Solution A*, and water (100:20:880)

**Standard solution A:** 0.01 mg/mL of 1,1-cyclobutanedicarboxylic acid in *Mobile phase*

**Standard solution B:** 5  $\mu$ g/mL of 1,1-cyclobutanedicarboxylic acid in *Mobile phase*

**System suitability solution:** 2.5  $\mu$ g/mL of 1,1-cyclobutanedicarboxylic acid and 0.5 mg/mL of carboplatin prepared as follows. Mix 1.0 mL of *Standard solution B* with 1.0 mL of *Standard solution* in the Assay.

**Sample solution:** Nominally equivalent to 1 mg/mL of carboplatin from the contents of one container diluted with *Mobile phase*. Complete the chromatographic analysis of the solution within 2 h.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.0-mm  $\times$  30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 100  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for carboplatin and 1,1-cyclobutanedicarboxylic acid are 0.65 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.5 between the carboplatin and 1,1-cyclobutanedicarboxylic acid peaks

$\Delta$ USP40

**Relative standard deviation:** NMT 10%

**Analysis**

**Samples:** *Standard solution A* and *Sample solution*

Calculate the percentage of 1,1-cyclobutanedicarboxylic acid in the portion of Carboplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of 1,1-cyclobutanedicarboxylic acid from the *Sample solution*

$r_S$  = peak response of 1,1-cyclobutanedicarboxylic acid from *Standard solution A*

$C_S$  = concentration of 1,1-cyclobutanedicarboxylic acid in *Standard solution A* (mg/mL)

$C_U$  = nominal concentration of carboplatin in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 1.0%

## SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.54 USP Endotoxin Units/mg of carboplatin
- **STERILITY TESTS** (71), *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Meets the requirements
- **CONSTITUTED SOLUTION**: At the time of use, it meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.
- **PH** (791)

**Sample solution:** Use Sterile Water for Injection, and constitute as directed in the labeling.

**Acceptance criteria:** 5.0–7.0

- **WATER DETERMINATION** (921), *Method I*

**Analysis:** Use anhydrous formamide as the extraction solvent. Introduce 50 mL of anhydrous formamide into the titration vessel, and titrate with the *Reagent* to the electrometric endpoint. Use the formamide thus dried to rinse a suitable glass syringe equipped with an 8-cm long, 22-gauge needle. Add the rinse back to the titration vessel, and titrate the vessel contents again, if necessary. Via the syringe, withdraw 5 mL of the formamide thus titrated and, through the closure of the container, expel the contents into the container. Shake the container to obtain a solution. With the same syringe, withdraw all of the contents of the container, and transfer to the titration vessel. Titrate to the endpoint, adjusting the feeding speed control to the lowest setting to avoid overtitration.

**Acceptance criteria:** NMT 3.0%

## ADDITIONAL REQUIREMENTS

## Change to read:

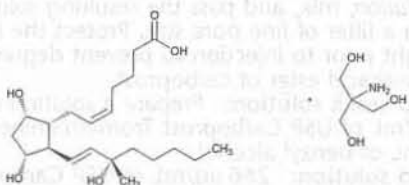
- **PACKAGING AND STORAGE:** • *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution*, • (CN 1-May-2017) and protect from light.



• **USP REFERENCE STANDARDS** (11)

USP Carboplatin RS  
USP Endotoxin RS

## Carboprost Tromethamine



$C_{21}H_{36}O_5 \cdot C_4H_{11}NO_3$  489.64

Prosta-5,13-dien-1-oic acid, 9,11,15-trihydroxy-15-methyl-, (5Z, 9α,11α,13E,15S)-, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1);

(Z)-7-[[1R,2R,3R,5S)-3,5-Dihydroxy-2-[(E)-(3S)-3-hydroxy-3-methyl-1-octenyl]cyclopentyl]-5-heptenoic acid compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1);

(15S)-15-Methylprostaglandin  $F_{2\alpha}$  tromethamine [58551-69-2].

### DEFINITION

Carboprost Tromethamine contains NLT 95.0% and NMT 105.0% of carboprost tromethamine ( $C_{21}H_{36}O_5 \cdot C_4H_{11}NO_3$ ), calculated on the dried basis.

**[CAUTION]**—Great care should be taken to prevent inhaling particles of Carboprost Tromethamine and exposing the skin to it.]

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Buffer:** 0.02 M monobasic potassium phosphate  
**Mobile phase:** Acetonitrile, methanol, and *Buffer* (20:30:50). Adjust with phosphoric acid to an apparent pH of 3.0.

**Diluent:** Acetonitrile, methanol, and water (20:30:50)

**Standard solution:** 1.0 mg/mL of USP Carboprost Tromethamine RS in *Diluent*

**Sample solution:** 1.0 mg/mL of Carboprost Tromethamine in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4-mm × 15-cm; 3-μm packing L1

**Column temperature:** 30 ± 2°

**Flow rate:** 0.8 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—See *Table 1* for the relative retention times of *trans*-carboprost and carboprost.]

#### Suitability requirements

**Resolution:** NLT 1.2 between *trans*-carboprost and carboprost

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 0.73%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carboprost tromethamine ( $C_{21}H_{36}O_5 \cdot C_4H_{11}NO_3$ ) in the portion of Carboprost Tromethamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Carboprost Tromethamine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carboprost Tromethamine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0% on the dried basis

### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.5%

#### • ORGANIC IMPURITIES

**Buffer, Mobile phase, and Diluent:** Prepare as directed in the Assay.

**Standard stock solution:** 1.0 mg/mL of USP

Carboprost Tromethamine RS in *Diluent*

**System suitability solution:** 0.1 mg/mL of 15-epi-carboprost in the *Standard stock solution*

**Standard solution:** 0.025 mg/mL of USP Carboprost Tromethamine RS in *Diluent* from the *Standard stock solution*

**Sensitivity solution:** 0.001 mg/mL of USP Carboprost Tromethamine RS in *Diluent* from the *Standard stock solution*

**Sample solution:** 1 mg/mL of Carboprost Tromethamine in *Diluent*

**Chromatographic system:** Proceed as directed in the Assay, except use an *Injection volume* of 20 μL.

#### System suitability

**Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—See *Table 1* for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 1.0 between 15-epicarboprost and *trans*-carboprost; NLT 1.2 between *trans*-carboprost and carboprost, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for carboprost, *Standard solution*

**Signal-to-noise ratio:** NLT 10 for carboprost, *Sensitivity solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Carboprost Tromethamine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of carboprost tromethamine from the *Standard solution*

$C_S$  = concentration of USP Carboprost Tromethamine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carboprost Tromethamine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 1*.



Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
15-Epicarboprost <sup>a</sup>	0.90	2.0
<i>trans</i> -Carboprost <sup>b</sup>	0.94	3.0
Carboprost	1.0	—
Any individual unspecified impurity	—	0.10
Total impurities	—	4.0

<sup>a</sup> (Z)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-[(*E*)-(3*R*)-3-hydroxy-3-methyloct-1-enyl]cyclopentyl]-5-heptenoic acid.

<sup>b</sup> (E)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-[(*E*)-(3*S*)-3-hydroxy-3-methyloct-1-enyl]cyclopentyl]-5-heptenoic acid.

### SPECIFIC TESTS

#### • OPTICAL ROTATION, Specific Rotation (7815)

**Sample solution:** 10 mg/mL of Carboprost Tromethamine in alcohol

**Acceptance criteria:** +18° to +24°

#### • LOSS ON DRYING (731)

**Analysis:** Dry under vacuum at a pressure not exceeding 5 mm of mercury at 50° for 16 h.

**Acceptance criteria:** NMT 1.0%

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE:

Preserve in well-closed containers, and store in a freezer.

#### • USP REFERENCE STANDARDS (11)

USP Carboprost Tromethamine RS

## Carboprost Tromethamine Injection

### DEFINITION

Carboprost Tromethamine Injection is a sterile solution of Carboprost Tromethamine in aqueous solution, which may also contain Benzyl Alcohol, Sodium Chloride, and Tromethamine. It contains NLT 90.0% and NMT 110.0% of the labeled amount of carboprost (C<sub>21</sub>H<sub>36</sub>O<sub>5</sub>).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Sample:** Extract the equivalent of 2.5 mg of carboprost tromethamine from a volume of Injection, with 1.5–2 times its volume of chloroform. Discard the chloroform layer, and acidify the aqueous layer with 3–5 drops of hydrochloric acid. Extract the acidified solution with an equivalent volume of chloroform. Filter the chloroform layer through a pledget of cotton, and concentrate it to a volume of less than 1 mL. Combine the resulting solution with 150–180 mg of potassium bromide. Dry the potassium bromide mixture in a vacuum overnight, and prepare a pellet from the dried mixture.

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Buffer:** Dissolve 10.5 g of citric acid in 75 mL of water. Adjust with 5 N sodium hydroxide to a pH of 4.0, and dilute with water to 100 mL.

**Mobile phase:** Methylene chloride, 1,3-butanediol, and water (992: 7: 0.5)

**Internal standard solution:** 3 mg/mL of guaifenesin in Mobile phase

**System suitability solution:** Transfer 5 mg of USP Carboprost Tromethamine RS to a stoppered, 50-mL centrifuge tube. Add 20.0 mL of methylene chloride and 2 mL of Buffer. Shake the stoppered tube for 10 min, and centrifuge. Remove and discard the top (aqueous) layer, and transfer a 4.0-mL aliquot of the lower (methylene chloride) layer to a suitable vial: Evap-

orate with the aid of a stream of nitrogen to dryness. Add 100 µL of a freshly prepared solution of α-bromo-2'-acetonaphthone in acetonitrile (1 in 50). Swirl to wash down the sides of the vial. Add 50 µL of a freshly prepared solution of diisopropylethylamine in acetonitrile (1 in 100), swirl again, and place the vial in a suitable heating device maintained at 30°–35° for NLT 15 min. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 2.0 mL of Internal standard solution, mix, and pass the resulting solution through a filter of fine pore size. Protect the filtrate from light prior to injection to prevent degradation of the naphthacyl ester of carboprost.

**Standard stock solution:** Prepare a solution containing 332 µg/mL of USP Carboprost Tromethamine RS and 9 mg/mL of benzyl alcohol.

**Standard solution:** 266 µg/mL of USP Carboprost Tromethamine RS, prepared as follows. Transfer 2.0 mL of Standard stock solution into a stoppered centrifuge tube. Add 20.0 mL of methylene chloride and 1.0 mL of Buffer, shake the stoppered tube for 10 min, and centrifuge. Remove and discard the top (aqueous) layer, transfer an 8.0-mL aliquot of the lower (methylene chloride) layer to a suitable vial, and evaporate the solution with the aid of a stream of nitrogen. [NOTE—The residue does not evaporate to dryness because of the presence of benzyl alcohol.] Add 100 µL of a freshly prepared solution of α-bromo-2'-acetonaphthone in acetonitrile (1 in 50), and swirl to wash down the sides of the vial. Add 50 µL of a freshly prepared solution of diisopropylethylamine in acetonitrile (1 in 100). Swirl again, and place the vial in a suitable heating device maintained at 30°–35° for NLT 15 min. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 1.0 mL of Internal standard solution, mix, and pass the resulting solution through a filter of fine pore size. Protect the filtrate from light prior to injection to prevent degradation of the naphthacyl ester of carboprost.

**Sample solution:** Nominally 200 µg/mL of carboprost, prepared as follows. Pipet a volume of Injection, equivalent to 500 µg of carboprost, to a stoppered, 50-mL centrifuge tube. Add 20.0 mL of methylene chloride and 1.0 mL of Buffer, shake the stoppered tube for 10 min, and centrifuge. Remove and discard the top (aqueous) layer, transfer an 8.0-mL aliquot of the lower (methylene chloride) layer to a suitable vial, and evaporate the solution with the aid of a stream of nitrogen. [NOTE—The residue does not evaporate to dryness because of the presence of benzyl alcohol.] Add 100 µL of a freshly prepared solution of α-bromo-2'-acetonaphthone in acetonitrile (1 in 50), and swirl to wash down the sides of the vial. Add 50 µL of a freshly prepared solution of diisopropylethylamine in acetonitrile (1 in 100). Swirl again, and place the vial in a suitable heating device maintained at 30°–35° for NLT 15 min. Evaporate the acetonitrile from the vial with the aid of a stream of nitrogen, add 1.0 mL of Internal standard solution, mix, and pass the resulting solution through a filter of fine pore size. Protect the filtrate from light prior to injection to prevent degradation of the naphthacyl ester of carboprost.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; 10-µm packing L3

**Flow rate:** 1.8 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** System suitability solution

[NOTE—The relative retention times for guaifenesin and 2-naphthacyl ester of carboprost are 0.6 and 1.0, respectively.]



**Suitability requirements**

**Resolution:** NLT 4.0 between guaifenesin and 2-naphthacyl ester of carboprost

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carboprost ( $C_{21}H_{36}O_5$ ) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak response ratio of the 2-naphthacyl ester of carboprost to the internal standard of the *Sample solution*

$R_S$  = peak response ratio of the 2-naphthacyl ester of carboprost to the internal standard of the *Standard solution*

$C_S$  = concentration of USP Carboprost Tromethamine RS in the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of carboprost in the *Sample solution* ( $\mu\text{g/mL}$ )

$M_{r1}$  = molecular weight of carboprost, 368.51

$M_{r2}$  = molecular weight of carboprost tromethamine, 489.64

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 714.3 USP Endotoxin Units/mg of carboprost tromethamine
- **PH (791):** 7.0–8.0
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, and store in a refrigerator.
- **USP REFERENCE STANDARDS (11)**  
USP Carboprost Tromethamine RS  
USP Endotoxin RS

**Carboxymethylcellulose Sodium**

Cellulose carboxymethyl ether sodium salt [9004-32-4].

**DEFINITION**

Carboxymethylcellulose Sodium is the sodium salt of a polycarboxymethyl ether of cellulose. It contains NLT 6.5% and NMT 9.5% of sodium (Na), calculated on the dried basis.

**IDENTIFICATION****• A. PROCEDURE**

**Sample solution:** Add 1 g of powdered Carboxymethylcellulose Sodium to 50 mL of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced.

**Analysis:** To 1 mL of the *Sample solution*, diluted with an equal volume of water in a small test tube, add 5 drops of 1-naphthol TS. Incline the test tube, and carefully introduce down the side of the tube 2 mL of sulfuric acid so that it forms a lower layer.

**Acceptance criteria:** A red-purple color develops at the interface.

**• B. PROCEDURE**

**Sample solution:** Use the *Sample solution* from Identification test A.

**Analysis:** To 5 mL of the *Sample solution*, add an equal volume of barium chloride TS.

**Acceptance criteria:** A fine, white precipitate is formed.

- **C. IDENTIFICATION TESTS—GENERAL, Sodium (191):** A portion of the *Sample solution* meets the requirements.

**Sample solution:** Use the *Sample solution* from Identification test A.

**ASSAY****• PROCEDURE**

**Sample solution:** Transfer to a beaker 500 mg of Carboxymethylcellulose Sodium, add 80 mL of glacial acetic acid, heat the mixture in a boiling water bath for 2 h, and cool to room temperature.

**Analysis:** Titrate the *Sample solution* with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 2.299 mg of Na.

**Acceptance criteria:** NLT 6.5% and NMT 9.5% of Na, on the dried basis

**IMPURITIES****Inorganic Impurities****Delete the following:**

- **HEAVY METALS, Method II (231):** NMT 20 ppm, adding 1 mL of hydroxylamine hydrochloride solution (1 in 5) to the solution of the residue. (Official 1-Jan-2018)

**SPECIFIC TESTS****• VISCOSITY—ROTATIONAL METHODS (912)**

**Analysis:** Determine the viscosity in a water solution at the concentration stated on the label. Using undried Carboxymethylcellulose Sodium, weigh the amount that, on the dried basis, will provide 200 g of solution of the stated concentration. Add the substance in small amounts to 180 mL of stirred water contained in a tared, wide-mouth bottle, continue stirring rapidly until the powder is well wetted, add sufficient water to make the mixture weigh 200 g, and allow to stand, with occasional stirring, until solution is complete. Adjust the temperature to  $25 \pm 0.2^\circ$ , and determine the viscosity, using a rotational type of viscometer, making certain that the system reaches equilibrium before taking the final reading.

**Acceptance criteria:** The viscosity of solutions of 2% or higher concentration is NLT 80.0% and NMT 120.0% of that stated on the label; the viscosity of solutions of less than 2% concentration is NLT 75.0% and NMT 140.0% of that stated on the label.

- **PH (791):** 6.5–8.5 in a solution (1 in 100)

- **LOSS ON DRYING (731):** Dry a sample at  $105^\circ$  for 3 h: it loses NMT 10.0% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate the viscosity in solutions of stated concentrations.

**Carboxymethylcellulose Sodium Paste****DEFINITION**

Carboxymethylcellulose Sodium Paste contains NLT 16.0% and NMT 17.0% of carboxymethylcellulose sodium.

**IDENTIFICATION****• A.**

**Sample solution:** Digest a quantity of Paste, equivalent to 1 g of carboxymethylcellulose sodium, with 50 mL of water until the solution is virtually complete. Filter.

**Analysis:** To 30 mL of the *Sample solution* add 3 mL of hydrochloric acid. Filter the solution, and save the filtrate for Identification test C.



Acceptance criteria: A white precipitate is formed.

- **B.**  
**Sample solution:** The remainder of the *Sample solution* prepared in *Identification test A*  
**Analysis:** To the *Sample solution* add an equal volume of barium chloride TS.  
**Acceptance criteria:** A fine, white precipitate is formed.
- **C. IDENTIFICATION TESTS—GENERAL, Sodium (191):** The filtrate from *Identification test A* meets the requirements of the tests.

## ASSAY

### PROCEDURE

**Sample:** 2 g

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N perchloric acid in dioxane VS

**Endpoint detection:** Potentiometric

**Analysis:** Transfer the *Sample* to a glass-stoppered, 250-mL conical flask. Add 75 mL of glacial acetic acid, attach a condenser, and reflux for 2 h. Cool, transfer the mixture to a 250-mL beaker with the aid of small volumes of glacial acetic acid. Titrate with *Titrant*. Each mL of 0.1 N perchloric acid is equivalent to 29.67 mg of carboxymethylcellulose sodium.

**Acceptance criteria:** 16.0%–17.0%

## IMPURITIES

**Delete the following:**

- **HEAVY METALS, Method II (231)**  
**Test preparation:** Prepare as directed in the chapter, using 400 mg of Paste and adding 1 mL of hydroxylamine hydrochloride solution (200 mg/mL) to the solution of the residue.  
**Acceptance criteria:** NMT 50 µg/g. (Official 1-Jan-2018)

## SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total bacterial count does not exceed  $10^3$  cfu/g, and the tests for *Salmonella* species and *Escherichia coli* are negative.
- **CONSISTENCY**  
**Apparatus:** A penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g, having a detachable steel tip of the following dimensions: the tip of the cone has an angle of 30°; the point of the tip is truncated to a diameter of  $0.381 \pm 0.025$  mm; the base of the tip is  $8.38 \pm 0.05$  mm in diameter; and the length of the tip is  $14.94 \pm 0.05$  mm.  
The remaining portion of the cone has an angle of 90°, is 28 mm in height, and has a maximum diameter at the base of 65 mm. The containers for the test are flat-bottom metal cylinders that are  $100 \pm 6$  mm in diameter and NLT 65 mm in height. They are constructed of at least 1.6-mm (16-gauge) metal, and are provided with well-fitting, water-tight covers.

**Sample:** Paste

**Analysis:** Place the required number of containers in an oven, bring them and a quantity of the *Sample* to a temperature of  $82 \pm 2.5^\circ$ , and pour the *Sample* into one or more of the containers, filling to within 6 mm of the rim. Cool to  $25 \pm 2.5^\circ$  over a period of NLT 16 h, protected from drafts. Two h before the test, place the containers in a water bath at  $25 \pm 0.5^\circ$ . If the room temperature is below  $23.5^\circ$  or above  $26.5^\circ$ , adjust the temperature of the cone to  $25 \pm 0.5^\circ$  by placing it in the water bath.

Without disturbing the surface of the substance under test, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the test substance at a spot 25–38 mm from the edge of the container. Adjust the zero setting and

quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration from the scale. Make three or more trials, each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the test substance for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of the three or more readings, and conduct further trials to a total of 10 if the individual results differ from the average by more than  $\pm 3\%$ .

**Acceptance criteria:** The final average of the trials is 30.0–36.0 mm, indicating a consistency value of between 300 and 360.

### LOSS ON DRYING (731)

**Analysis:** Dry at  $105^\circ$  for 3 h.

**Acceptance criteria:** NMT 2.0%

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and avoid prolonged exposure to temperatures exceeding  $30^\circ$ .

## Carboxymethylcellulose Sodium Tablets

» Carboxymethylcellulose Sodium Tablets contain an amount of sodium (Na) equivalent to not less than 6.5 percent and not more than 9.5 percent of the labeled amount of carboxymethylcellulose sodium.

**Packaging and storage—**Preserve in tight containers.

**Identification—**Digest a quantity of powdered Tablets, equivalent to about 1 g of carboxymethylcellulose sodium, with 50 mL of water until solution is virtually complete, and filter: the filtrate responds to the following tests.

**A:** To about 30 mL of the solution add 3 mL of hydrochloric acid: a white precipitate is formed.

**B:** To the remainder of the solution add an equal volume of barium chloride TS: a fine, white precipitate is formed.

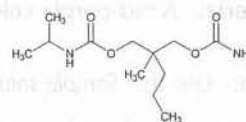
**C:** The filtrate from *Identification test A* responds to the tests for Sodium (191).

**Disintegration (701):** 2 hours.

**Uniformity of dosage units (905):** meet the requirements.

**Assay—**Weigh and finely powder not less than 20 Tablets. Weigh accurately a portion of the powder, equivalent to about 500 mg of carboxymethylcellulose sodium, add 80 mL of glacial acetic acid, heat the mixture on a steam bath for 2 hours, cool to room temperature, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 2.299 mg of Na.

## Carisoprodol



$C_{12}H_{24}N_2O_4$  260.33  
 (±)-2-Methyl-2-propyl-1,3-propanediol carbamate isopropyl-carbamate [78-44-4].



**DEFINITION**

Carisoprodol contains NLT 98.0% and NMT 102.0% of  $C_{12}H_{24}N_2O_4$ , calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (177K)**
- **B.** The retention time of the major peak in the *Sample solution* corresponds to that in the *Standard solution* as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

Diluent: Acetonitrile and water (50:50)  
 Solution A: Acetonitrile and water (25:75)  
 Solution B: Acetonitrile  
 Mobile phase: See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35	100	0
36	80	20
51	80	20
52	100	0
60	100	0

*System suitability solution*: 0.125 mg/mL each of USP Carisoprodol Related Compound A RS, USP Meprobamate RS, and USP Carisoprodol RS in *Diluent*

*Standard solution*: 2.5 mg/mL of USP Carisoprodol RS in *Diluent*

*Sample solution*: 2.5 mg/mL of Carisoprodol in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 200 nm

Column: 4.6-mm × 15-cm; 4-μm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection size: 25 μL

**System suitability**

*Samples*: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

**Suitability requirements**

**Resolution**: NLT 1.5 between carisoprodol related compound A and meprobamate, *System suitability solution*

**Tailing factor**: NMT 2.5 for the carisoprodol peak, *Standard solution*

**Relative standard deviation**: NMT 2.0% for the carisoprodol peak, *Standard solution*

**Analysis**

*Samples*: *Standard solution* and *Sample solution*

Calculate the percentage of carisoprodol ( $C_{12}H_{24}N_2O_4$ ) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of carisoprodol from the *Sample solution*

$r_S$  = peak response of carisoprodol from the *Standard solution*

$C_S$  = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carisoprodol in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION (281)**: NMT 0.1%

**Delete the following:**

- **HEAVY METALS, Method II (231)**: NMT 10 ppm (Official 1-Jan-2018)

• **ORGANIC IMPURITIES**

*Diluent*, *Mobile phase*, *System suitability solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.

*Standard solution*: 10 μg/mL of USP Carisoprodol RS in *Diluent*

*Sample solution*: 50 mg/mL of Carisoprodol in *Diluent*. [NOTE—Sonication may be used to aid dissolution.]

**System suitability**

*Samples*: *System suitability solution* and *Standard solution*

[NOTE—See *Table 2* for the relative retention times.]

**Suitability requirements**

**Resolution**: NLT 1.5 between carisoprodol related compound A and meprobamate, *System suitability solution*

**Tailing factor**: NMT 2.5 for the carisoprodol peak, *Standard solution*

**Relative standard deviation**: NMT 5.0% for the carisoprodol peak, 3 replicate injections of *Standard solution*

**Analysis**

*Samples*: *Standard solution* and *Sample solution*

Identify the specified impurities using the relative retention times given in *Table 2*.

Calculate the percentage of each impurity in the portion of Carisoprodol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of the impurity from the *Sample solution*

$r_S$  = peak response of carisoprodol from the *Standard solution*

$C_S$  = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carisoprodol in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Carisoprodol related compound A <sup>a</sup>	0.19	0.06	0.1
Meprobamate	0.24	0.08	0.5
Carisoprodol monocarbamate <sup>b</sup>	0.86	1.4	0.1
Carisoprodol	1.0	—	—
Any other unknown individual impurity	—	1.0	0.1
Total impurities	—	—	1.0

<sup>a</sup> 2-Hydroxymethyl-2-methylpentyl carbamate.

<sup>b</sup> N-Isopropyl-2-hydroxymethyl-2-methylpentyl carbamate.

**SPECIFIC TESTS**

- **LOSS ON DRYING (731)**: Dry a sample in vacuum at 60° for 3 h: it loses NMT 0.5% of its weight.



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers at room temperature.
- **USP REFERENCE STANDARDS (11)**
  - USP Carisoprodol RS
  - USP Carisoprodol Related Compound A RS
  - 2-Hydroxymethyl-2-methylpentyl carbamate.
  - $C_8H_{17}NO_3$  175.23
  - USP Meprobamate RS

**Carisoprodol Tablets****DEFINITION**

Carisoprodol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carisoprodol ( $C_{12}H_{24}N_2O_4$ ).

**IDENTIFICATION**

- **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Diluent:** Acetonitrile and water (50:50)

**Mobile phase:** Acetonitrile and water (25:75)

**System suitability solution:** 0.1 mg/mL each of USP Carisoprodol Related Compound A RS, USP Meprobamate RS, and USP Carisoprodol RS in *Diluent*

**Standard solution:** 2.5 mg/mL of USP Carisoprodol RS in *Diluent*

**Sample solution:** Nominally 2.5 mg/mL in *Diluent* prepared as follows. Transfer an amount equivalent to the label claim of carisoprodol from powdered Tablets (NLT 20) to a suitable volumetric flask, and fill 50% of the flask volume with *Diluent*. Place in an ultrasonic bath for 30 min, and shake mechanically for 60 min. Dilute with *Diluent* to volume. Pass a portion of this solution through a suitable membrane filter, and use the filtrate as the *Sample solution*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Column:** 4.6-mm × 15 cm; 4-μm packing L1

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection size:** 25 μL

**Run time:** 1.5 times the retention time of carisoprodol

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between carisoprodol related compound A and meprobamate, *System suitability solution*

**Tailing factor:** NMT 2.5 for the carisoprodol peak, *Standard solution*

**Relative standard deviation:** NMT 2.0% for the carisoprodol peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carisoprodol ( $C_{12}H_{24}N_2O_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of carisoprodol from the *Sample solution*

$r_s$  = peak response of carisoprodol from the *Standard solution*

$C_s$  = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of carisoprodol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION (711)**

**Medium:** 0.05 M phosphate buffer, pH 6.9 (see *Reagents, Indicators, and Solutions—Buffer Solutions*) containing 5 units of α-amylase per mL; 900 mL

[NOTE—Use only freshly prepared solutions containing α-amylase; and equilibrate the *Medium* at 37° for NMT 1 h before beginning the *Dissolution* test.]

**Apparatus 2:** 75 rpm

**Time:** 60 min

**Mobile phase:** Acetonitrile and water (40:60)

**System suitability solution:** 2.4 mg/mL of 2-methyl-2-propyl-1,3-propanediol and 3.4 mg/mL of USP Carisoprodol RS in *Mobile phase*

**Standard solution:** 0.4 mg/mL of USP Carisoprodol RS in *Medium*

[NOTE—A volume of acetonitrile not exceeding 2% of the final total volume of solution may be used to aid in dissolving the carisoprodol.]

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 3.9-mm × 30-cm; packing L1

**Temperature:** 30 ± 1° for column and detector

**Flow rate:** 2 mL/min

**Injection size:** 150 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for 2-methyl-2-propyl-1,3-propanediol and carisoprodol are about 0.5 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between 2-methyl-2-propyl-1,3-propanediol and carisoprodol, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for 3 replicate injections of the *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Record the peak responses, and measure the heights for the major peaks.

Calculate the percentage of the labeled amount of carisoprodol ( $C_{12}H_{24}N_2O_4$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times (C_s/L) \times V \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Carisoprodol RS in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of carisoprodol ( $C_{12}H_{24}N_2O_4$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements



**IMPURITIES****• ORGANIC IMPURITIES**

**Diluent, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution:** 10 µg/mL of USP Carisoprodol RS in Diluent

**Sample solution:** Nominally 10 mg/mL in Diluent prepared as follows. Transfer an amount equivalent to four times the label claim of carisoprodol from powdered Tablets (NLT 20 Tablets) to a suitable volumetric flask, and fill 50% of the flask volume with Diluent. Place in an ultrasonic bath for 30 min, and shake mechanically for 60 min. Dilute with Diluent to volume. Pass a portion of this solution through a suitable membrane filter, and use the filtrate as the Sample solution.

**System suitability**

**Samples:** System suitability solution and Standard solution

[NOTE—See Table 1 for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between carisoprodol related compound A and meprobamate, System Suitability solution

**Tailing factor:** NMT 2.5 for the carisoprodol peak, Standard solution

**Relative standard deviation:** NMT 5.0% for the carisoprodol peak for 3 replicate injections of the Standard solution

**Analysis**

**Samples:** Standard solution and Sample solution

Identify the specified impurities using the relative retention times given in Table 1.

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of the impurity from the Sample solution

$r_S$  = peak response of carisoprodol from the Standard solution

$C_S$  = concentration of USP Carisoprodol RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of carisoprodol in the Sample solution (mg/mL)

$F$  = relative response factor (see Table 1).

**Acceptance criteria:** See Table 1.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Carisoprodol related compound A <sup>a</sup>	0.19	0.06	0.75
Meprobamate	0.24	0.08	0.65
Carisoprodol monocarbamate <sup>b</sup>	0.86	1.4	0.20
Carisoprodol	1.0	—	—
Any other unknown degradation product	—	1.0	0.20
Total impurities	—	—	1.25

<sup>a</sup> 2-Hydroxymethyl-2-methylpentyl carbamate.

<sup>b</sup> N-Isopropyl-2-hydroxymethyl-2-methylpentyl carbamate.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers at controlled room temperature.

**• USP REFERENCE STANDARDS (11)**

USP Carisoprodol RS

USP Carisoprodol Related Compound A RS

2-Hydroxymethyl-2-methylpentyl carbamate.

$C_8H_{17}NO_3$  175.23

USP Meprobamate RS

**Carisoprodol and Aspirin Tablets****DEFINITION**

Carisoprodol and Aspirin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of carisoprodol ( $C_{12}H_{24}N_2O_4$ ) and aspirin ( $C_9H_8O_4$ ).

**IDENTIFICATION**

- A.** The retention times of aspirin and carisoprodol from the Sample solution correspond to those of Standard solution A, as obtained in the Assay.

**ASSAY****• PROCEDURE**

**Buffer:** Combine 5 mL of glacial acetic acid and 500 mL of water, and pass the mixture through a membrane filter of 0.5-µm or finer pore size. Use the filtrate.

**Mobile phase:** Methanol and Buffer (64:36)

**Diluent:** Acetonitrile, glacial acetic acid, and water (40:1:59)

**Standard solution A:** USP Reference Standards in Diluent as listed below and prepared as follows. Transfer 80 mg of USP Aspirin RS and 80 mg of USP Carisoprodol RS to a 25-mL volumetric flask. Add 15 mL of Diluent, swirl for 5 min, and sonicate for 25–30 s. Dilute with Diluent to volume.

**Aspirin:** 3.2 mg/mL of USP Aspirin RS

**Carisoprodol:** 3.2 mg/mL of USP Carisoprodol RS, where  $J$  is the ratio of the labeled amount, in mg, of carisoprodol to the labeled amount of aspirin

**Standard solution B:** 0.016 mg/mL of USP Salicylic Acid RS in Diluent

**System suitability solution:** 0.5 mg/mL of salicylic acid in Standard solution A

**Sample solution:** Nominally 3.25 mg/mL of aspirin prepared as follows. Finely powder NLT 20 Tablets. Transfer a portion of powder, equivalent to 325 mg of aspirin, to a 100-mL volumetric flask. Add 50 mL of Diluent, and swirl for 5 min. Sonicate for 25–30 s, shake by mechanical means for 30 min, and dilute with Diluent to volume. Pass a portion of this solution through a membrane filter of 0.5-µm or finer pore size, and use the filtrate within 8 h.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector**

**Aspirin and carisoprodol:** Refractive index

**Salicylic acid:** UV 313 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Temperatures**

**Refractive index detector:** 30 ± 1°

**Column:** 30 ± 1°

**Flow rate:** 1 mL/min

**Injection volume:** 50 µL

**System suitability**

**Samples:** Standard solution A, Standard solution B, and System suitability solution

[NOTE—The relative retention times for aspirin, salicylic acid, and carisoprodol are about 0.6, 0.7, and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.2 between solvent and aspirin;

NLT 1.5 between aspirin and salicylic acid, System suitability solution using the refractive index detector



**Relative standard deviation:** NMT 2.0% for *Standard solution A* using the refractive index detector; NMT 5.0% for *Standard solution B* at 313 nm

#### Analysis

**Samples:** *Standard solution A* and *Sample solution*  
Calculate the percentages of the labeled amounts of aspirin ( $C_9H_8O_4$ ) and carisoprodol ( $C_{12}H_{24}N_2O_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of aspirin or carisoprodol from the *Sample solution*

$r_s$  = peak response of aspirin or carisoprodol from the *Standard solution A*

$C_s$  = concentration of USP Aspirin RS or USP Carisoprodol RS in *Standard solution A* (mg/mL)

$C_u$  = nominal concentration of aspirin or carisoprodol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amounts of carisoprodol ( $C_{12}H_{24}N_2O_4$ ) and aspirin ( $C_9H_8O_4$ )

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 45 min

**Buffer:** Glacial acetic acid in water (1 in 50)

**Mobile phase:** Methanol and Buffer (51:49)

**Standard solution:** USP Reference Standards as listed below and prepared as follows. Transfer 90 mg of USP Aspirin RS and 90 mg of USP Carisoprodol RS to a 250-mL volumetric flask. Add 5 mL of acetonitrile, previously passed through a membrane filter of 0.5- $\mu$ m or finer pore size, and swirl to dissolve. Dilute with water to volume.

**Aspirin:** 0.36 mg/mL of USP Aspirin RS

**Carisoprodol:** 0.36 mg/mL of USP Carisoprodol RS, where  $j$  is the ratio of the labeled amount, in mg, of carisoprodol to the labeled amount of aspirin

**System suitability solution:** 0.36 mg/mL of salicylic acid in the *Standard solution*

**Sample solution:** Filter a portion of the solution under test. Use the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Temperatures**

**Detector:** 30  $\pm$  1°

**Column:** 30  $\pm$  1°

**Flow rate:** 2 mL/min

**Injection volume:** 300  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for aspirin and carisoprodol are 0.4 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between aspirin and salicylic acid; NLT 1.5 between carisoprodol and salicylic acid, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of aspirin ( $C_9H_8O_4$ ) and carisoprodol ( $C_{12}H_{24}N_2O_4$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times (1/L) \times 100$$

$r_u$  = peak response of aspirin or carisoprodol from the *Sample solution*

$r_s$  = peak response of aspirin or carisoprodol from the *Standard solution*

$C_s$  = concentration of USP Aspirin RS or USP Carisoprodol RS in the *Standard solution* (mg/mL)

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim of aspirin or carisoprodol (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amounts of aspirin ( $C_9H_8O_4$ ) and carisoprodol ( $C_{12}H_{24}N_2O_4$ ) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Content Uniformity* with respect to aspirin and to carisoprodol

#### IMPURITIES

##### • LIMIT OF FREE SALICYLIC ACID

**Mobile phase, Diluent, Standard solution B, System suitability solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

#### Analysis

**Samples:** *Standard solution B* and *Sample solution*  
Calculate the percentage of free salicylic acid in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of salicylic acid from the *Sample solution*

$r_s$  = peak response of salicylic acid from *Standard solution B*

$C_s$  = concentration of USP Salicylic Acid RS in *Standard solution B* (mg/mL)

$C_u$  = nominal concentration of aspirin in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 3.0% of free salicylic acid

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

##### • USP REFERENCE STANDARDS (11)

USP Aspirin RS

USP Carisoprodol RS

USP Salicylic Acid RS

## Carisoprodol, Aspirin, and Codeine Phosphate Tablets

#### DEFINITION

Carisoprodol, Aspirin, and Codeine Phosphate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of carisoprodol ( $C_{12}H_{24}N_2O_4$ ), aspirin ( $C_9H_8O_4$ ), and codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot 1/2H_2O$ ).

#### IDENTIFICATION

- **A.** The retention times of the aspirin, carisoprodol, and codeine phosphate peaks of the *Sample solutions* correspond to those of the *Standard solutions* obtained as directed in the *Assay for Aspirin and Carisoprodol* and the *Assay for Codeine Phosphate*.

#### ASSAY

##### • ASPIRIN AND CARISOPRODOL

**Buffer:** Combine 5 mL of glacial acetic acid and 500 mL of water, and pass the mixture through a membrane filter of 0.5- $\mu$ m or finer pore size. Use the filtrate.

**Mobile phase:** Methanol and Buffer (64:36)

**Diluent:** Acetonitrile, glacial acetic acid, and water (40:1:59)



**Standard solution A:** USP Reference Standards in *Diluent* as listed below and prepared as follows. Transfer 80 mg of USP Aspirin RS and 80 mg of USP Carisoprodol RS to a 25-mL volumetric flask. Add 15 mL of *Diluent*, swirl for 5 min, and sonicate for 25–30 s. Dilute with *Diluent* to volume.

**Aspirin:** 3.2 mg/mL of USP Aspirin RS

**Carisoprodol:** 3.2 mg/mL of USP Carisoprodol RS, where *J* is the ratio of the labeled amount, in mg, of carisoprodol to the labeled amount of aspirin

**Standard solution B:** 0.016 mg/mL of USP Salicylic Acid RS in *Diluent*

**System suitability solution:** 0.5 mg/mL of salicylic acid in *Standard solution A*

**Sample solution:** Nominally 3.25 mg/mL of aspirin from NLT 20 Tablets prepared as follows. Finely powder NLT 20 Tablets. Transfer a portion of powder, equivalent to 325 mg of aspirin, to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and swirl for 5 min. Sonicate for 25–30 s, shake by mechanical means for 30 min, and dilute with *Diluent* to volume. Pass a portion of this solution through a membrane filter of 0.5- $\mu$ m or finer pore size, and use the filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

#### Detector

**Aspirin and carisoprodol:** Refractive index

**Salicylic acid:** UV 313 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L7

#### Temperatures

**Refractive index detector:** 30  $\pm$  1°

**Column:** 30  $\pm$  1°

**Flow rate:** 1 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Samples:** *Standard solution A*, *Standard solution B*, and *System suitability solution*

[NOTE—The relative retention times for aspirin, salicylic acid, and carisoprodol are about 0.6, 0.7, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.2 between the solvent and aspirin peaks; NLT 1.5 between aspirin and salicylic acid, *System suitability solution* using the refractive index detector

**Relative standard deviation:** NMT 2.0% for *Standard solution A* using the refractive index detector; NMT 5.0% for *Standard solution B* at 313 nm

#### Analysis

**Samples:** *Standard solution A* and *Sample solution*  
Calculate the percentages of the labeled amounts of aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>) and carisoprodol (C<sub>12</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response of aspirin or carisoprodol from the *Sample solution*

*r<sub>S</sub>* = peak response of aspirin or carisoprodol from *Standard solution A*

*C<sub>S</sub>* = concentration of USP Aspirin RS or USP Carisoprodol RS in the *Standard solution A* (mg/mL)

*C<sub>U</sub>* = nominal concentration of aspirin or carisoprodol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% of the labeled amounts of aspirin (C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>) and carisoprodol (C<sub>12</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>)

#### • CODEINE PHOSPHATE

**Solution A:** 3.7 g/L of docusate sodium in methanol

**Solution B:** 2 g/L of ammonium nitrate in water

**Mobile phase:** *Solution A* and *Solution B* (60:40) adjusted with glacial acetic acid to a pH of 3.3  $\pm$  0.05

**Diluent:** Methanol and 0.01 N sulfuric acid (50:50)

**System suitability solution:** 0.16 mg/mL of USP Codeine Phosphate RS and 0.12 mg/mL of USP Codeine N-Oxide RS in *Diluent*

**Standard solution:** USP Reference Standards in *Diluent* as listed below. Swirl for 5 min, and sonicate for 25–30 s.

**Codeine phosphate:** 0.16 mg/mL of USP Codeine Phosphate RS

**Aspirin:** 0.16 mg/mL of USP Aspirin RS, where *J* is the ratio of the labeled amount, in mg, of aspirin to that of codeine phosphate

**Sample solution:** Nominally 0.16 mg/mL of codeine phosphate prepared as follows. Finely powder NLT 20 Tablets. Transfer an amount of powder equivalent to 16 mg of codeine phosphate to a 100-mL volumetric flask. Add 50 mL of *Diluent*, sonicate for 30 min, shake by mechanical means for 30 min, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 50  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for codeine N-oxide and codeine phosphate are 0.9 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.2 between codeine phosphate and codeine N-oxide, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of codeine phosphate (C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> · H<sub>3</sub>PO<sub>4</sub> · 1/2H<sub>2</sub>O) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of the *Sample solution* (mg/mL)

*M<sub>r1</sub>* = molecular weight of codeine phosphate hemihydrate, 406.37

*M<sub>r2</sub>* = molecular weight of anhydrous codeine phosphate, 397.37

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of codeine phosphate (C<sub>18</sub>H<sub>21</sub>NO<sub>3</sub> · H<sub>3</sub>PO<sub>4</sub> · 1/2H<sub>2</sub>O)

#### PERFORMANCE TESTS

##### • DISSOLUTION <711>

**Medium:** Water; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 45 min

##### Procedure for aspirin and carisoprodol

**Buffer:** Glacial acetic acid in water (1 in 50)

**Mobile phase:** Methanol and *Buffer* (51:49)

**Standard solution:** USP Reference Standards as listed below and prepared as follows. Transfer 90 mg of USP Aspirin RS and 90 mg of USP Carisoprodol RS to a 250-mL volumetric flask. Add 5 mL of acetonitrile, previously passed through a membrane filter of 0.5- $\mu$ m or finer pore size, and swirl to dissolve. Dilute with water to volume.



Aspirin: 0.36 mg/mL of USP Aspirin RS

Carisoprodol: 0.36/ mg/mL of USP Carisoprodol RS, where  $j$  is the ratio of the labeled amount, in mg, of carisoprodol to the labeled amount of aspirin

System suitability solution: 0.36 mg/mL of salicylic acid in the *Standard solution*

Sample solution: Pass a portion of the solution under test through a suitable filter, and use the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 3.9-mm  $\times$  30-cm; packing L1

Temperatures

Detector:  $30 \pm 1^\circ$

Column:  $30 \pm 1^\circ$

Flow rate: 2 mL/min

Injection volume: 300  $\mu$ L

#### System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for aspirin and carisoprodol are 0.4 and 1.0, respectively.]

#### Suitability requirements

Resolution: NLT 1.5 between aspirin and salicylic acid; NLT 1.5 between carisoprodol and salicylic acid, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of aspirin ( $C_9H_8O_4$ ) and carisoprodol ( $C_{12}H_{24}N_2O_4$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times V \times (1/L) \times 100$$

$r_u$  = peak response of aspirin or carisoprodol from the *Sample solution*

$r_s$  = peak response of aspirin or carisoprodol from the *Standard solution*

$C_s$  = concentration of USP Aspirin RS or USP Carisoprodol RS in the *Standard solution* (mg/mL)

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim of aspirin or carisoprodol (mg/ Tablet)

#### Procedure for codeine phosphate

Buffer: 4.0 g/L of docusate sodium and 1.5 g/L of ammonium nitrate in water

Mobile phase: Acetonitrile and *Buffer* (45:55)

Standard solution: 0.018 mg/mL of USP Codeine Phosphate RS in water

Sample solution: Pass a portion of the solution under test through a suitable filter, and use the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm  $\times$  30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 50  $\mu$ L

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

$r_u$  = peak response of the *Sample solution*

$r_s$  = peak response of the *Standard solution*

$C_s$  = concentration of USP Codeine Phosphate RS in the *Standard solution* (mg/mL)

$M_{r1}$  = molecular weight of codeine phosphate hemihydrate, 406.37

$M_{r2}$  = molecular weight of anhydrous codeine phosphate, 397.37

$V$  = volume of the *Medium*, 900 mL

$L$  = label claim of codeine phosphate (mg/Tablet)

Tolerances: NLT 75% (Q) of the labeled amounts of aspirin ( $C_9H_8O_4$ ), carisoprodol ( $C_{12}H_{24}N_2O_4$ ), and codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements for *Content Uniformity* with respect to aspirin, carisoprodol, and codeine phosphate

#### IMPURITIES

##### • ORGANIC IMPURITIES

Limit of free salicylic acid

Mobile phase, Diluent, *Standard solution B*, *System suitability solution*, *Sample solution*, *Chromatographic system*, and *System suitability*: Proceed as directed in the *Assay for Aspirin and Carisoprodol*.

#### Analysis

Samples: *Standard solution B* and *Sample solution*

Calculate the percentage of free salicylic acid in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of salicylic acid from the *Sample solution*

$r_s$  = peak response of salicylic acid from *Standard solution B*

$C_s$  = concentration of USP Salicylic Acid RS in *Standard solution B* (mg/mL)

$C_u$  = nominal concentration of aspirin in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 3.0% of free salicylic acid

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

##### • USP REFERENCE STANDARDS (11)

USP Aspirin RS

USP Carisoprodol RS

USP Codeine *N*-Oxide RS

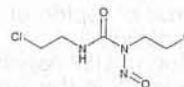
7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -ol *N*-oxide.

$C_{18}H_{21}NO_4$  315.37

USP Codeine Phosphate RS

USP Salicylic Acid RS

## Carmustine



$C_5H_9Cl_2N_3O_2$

Urea, *N,N'*-bis(2-chloroethyl)-*N*-nitroso-

1,3-Bis(2-chloroethyl)-1-nitrosourea [154-93-8].

214.05

#### DEFINITION

Carmustine contains NLT 98.0% and NMT 102.0% of  $C_5H_9Cl_2N_3O_2$ , calculated on the anhydrous and solvent-free basis.

[CAUTION—Use appropriate surgical gloves, arm covers, and a dust mask. Perform all work under a fume hood approved for testing cytotoxic agents when possible.]



**IDENTIFICATION****A. INFRARED ABSORPTION (197F)**

**Sample:** Melt a small portion of the sample in a suitable container in a controlled water bath or oven, and set the temperature between 33° and 40°.

**Standard:** A similar preparation of USP Carmustine RS

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****PROCEDURE**

[NOTE—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

**Mobile phase:** Acetonitrile and water (3:7)

**Standard solution:** 1.5 mg/mL of USP Carmustine RS in acetonitrile

**Sample solution:** 1.5 mg/mL of Carmustine in acetonitrile

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Refrigerated autosampler temperature:** 4°–5°

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 10 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.9

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of  $C_5H_9Cl_2N_3O_2$  in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Carmustine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carmustine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis

**IMPURITIES****Inorganic Impurities****Delete the following:**

- **HEAVY METALS, Method II (231):** NMT 20 ppm • (Official 1-Jan-2018)

**Organic Impurities****PROCEDURE 1: LIMIT OF ETHER-INSOLUBLE SUBSTANCES**

[NOTE—Perform in a well-ventilated fume hood.]

**Analysis:** Transfer 1.0 g of sample to a suitable container containing 10 mL of anhydrous ether, stir for 5 min, and immediately filter through a tared glass-filtering crucible of medium pore size. Wash the container with an additional 10 mL of ether, and filter through the same glass-filtering crucible. Dry the crucible at 105° for 1 h. Cool in a desiccator and weigh.

**Acceptance criteria:** The weight of the residue does not exceed 0.1%.

**PROCEDURE 2: LIMIT OF CARMUSTINE RELATED COMPOUND A**

[NOTE—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

**Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**Carmustine standard solution:** Use the *Standard solution*, prepared as directed in the Assay.

**Standard stock solution:** 0.75 mg/mL of USP Carmustine Related Compound A RS in acetonitrile

**Standard solution:** 0.0075 mg/mL of USP Carmustine Related Compound A RS in acetonitrile, from the *Standard stock solution*

**System suitability solution 1:** 0.75 μg/mL of USP Carmustine Related Compound A RS in acetonitrile, from the *Standard solution*

**System suitability solution 2:** Transfer 5.0 mL of *Carmustine standard solution* and 10.0 mL of *Standard stock solution* into a 100-mL volumetric flask, and dilute with acetonitrile to volume. Transfer 5.0 mL of this solution into a 50-mL volumetric flask, and dilute with acetonitrile to volume.

**System suitability**

**Samples:** *Carmustine standard solution*, *System suitability solution 1*, and *System suitability solution 2*

[NOTE—The relative retention times for carmustine related compound A and carmustine are 0.3 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 10 between carmustine related compound A and carmustine, *System suitability solution 2*

**Tailing factor:** NMT 1.9, *Carmustine standard solution*

**Relative standard deviation:** NMT 5%, *System suitability solution 1*

**Analysis**

[NOTE—Run the *Sample solution* at least 1.5 times the retention time of carmustine.]

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of carmustine related compound A in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of carmustine related compound A from the *Sample solution*

$r_S$  = peak response of carmustine related compound A from the *Standard solution*

$C_S$  = concentration of carmustine related compound A in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carmustine in the *Sample solution* (mg/mL)

Calculate the percentage of each unspecified impurity in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of any unspecified impurity from the *Sample solution*

$r_T$  = sum of all peak responses from the *Sample solution*

**Acceptance criteria**

Carmustine related compound A: NMT 0.5%

Any unspecified impurity: NMT 0.1%

**PROCEDURE 3: LIMIT OF 2-CHLOROETHYLAMINE**

[NOTE—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

**Standard solution 1 (0.2%):** 1.2 mg/mL of 2-chloroethylamine monohydrochloride in methanol. [NOTE—1.2 mg/mL of 2-chloroethylamine monohydrochloride is equivalent to 0.8 mg/mL of 2-chloroethylamine.]

**Standard solution 2 (0.1%):** 0.4 mg/mL of USP Carmustine RS in methanol

**Sample solution:** 0.4 g/mL of Carmustine in methanol

**Chromatographic system**  
(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic plate (20-cm × 20-cm) coated with silica gel 60



Application volume: 1  $\mu$ L  
 Developing solvent system 1: Ethyl acetate  
 Developing solvent system 2: Ethyl acetate and methanol (7:3)  
 Spray reagent 1: Diethylamine  
 Spray reagent 2: 0.1 N silver nitrate solution

**Analysis**

Samples: *Standard solution 1* (0.2%), *Standard solution 2* (0.1%), and *Sample solution*  
 Develop with *Developing solvent system 1* for 27 min, followed by air drying for 5 min. Develop again in *Developing solvent system 2* for 8 min, followed by air drying for 10 min. Spray the plate with *Spray reagent 1*, and heat the plate for 20 min in an oven at 100°. Allow the plates to cool to room temperature, and spray the plate with *Spray reagent 2*. Allow the plate to be exposed to UV light at 365 nm for 15 min. Examine the plate under UV light.

**Acceptance criteria**

**2-Chloroethylamine:** The spot for 2-chloroethylamine from the *Sample solution* is not more intense than the principal spot from *Standard solution 1* (0.2%).

**Any unspecified impurity:** Any spot if present in the chromatogram from the *Sample solution*, except the principal spot of carmustine and the spot of 2-chloroethylamine, is not more intense than the principal spot from *Standard solution 2* (0.1%).

• **PROCEDURE 4: LIMIT OF 2-CHLOROETHANOL**

**Standard solution:** 0.02 mg/mL of 2-chloroethanol in acetonitrile

**System suitability solution:** 0.01 mg/mL of 2-chloroethanol in acetonitrile, diluted from the *Standard solution*

**Sample solution:** 10 mg/mL of Carmustine in acetonitrile. [NOTE—Prepare in low-actinic glassware, and keep refrigerated until use.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 30-m  $\times$  0.53-mm column bonded with a 1- $\mu$ m film of phase G16

**Temperature**

**Injector:** 90°

**Detector:** 260°

**Column:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	6
40	30	80	14
80	30	200	3

**Carrier gas:** Helium

**Flow rate:** 7 mL/min

**Injection size:** 5  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Relative standard deviation:** NMT 5%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 2-chloroethanol in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of 2-chloroethanol from the *Sample solution*

$r_S$  = peak response of 2-chloroethanol from the *Standard solution*

$C_S$  = concentration of 2-chloroethanol in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carmustine in the *Sample solution* (mg/mL)

**Acceptance criteria**

**2-Chloroethanol:** NMT 0.1%

• **PROCEDURE 5: LIMIT OF ACETALDEHYDE**

**Standard solution:** 10  $\mu$ g/mL of acetaldehyde in acetonitrile

**Sample solution:** 10 mg/mL of Carmustine in acetonitrile. [NOTE—Prepare in low-actinic glassware, and keep refrigerated until use.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 30-m  $\times$  0.53-mm column bonded with a 5- $\mu$ m film of phase G1

**Temperature**

**Injector:** 70°

**Detector:** 260°

**Column:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	0	40	6
40	30	210	3

**Injector split ratio:** 15:1

**Carrier gas:** Helium

**Flow rate:** 3 mL/min

**Injection size:** 5  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 5%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of acetaldehyde in the portion of Carmustine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of acetaldehyde from the *Sample solution*

$r_S$  = peak response of acetaldehyde from the *Standard solution*

$C_S$  = concentration of acetaldehyde in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carmustine in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Acetaldehyde:** NMT 0.1%

**SPECIFIC TESTS**

• **WATER DETERMINATION, Method I (921):** NMT 0.5%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature between 2° and 8°.

• **USP REFERENCE STANDARDS (11)**

USP Carmustine RS

USP Carmustine Related Compound A RS

1,3-Bis(2-chloroethyl) urea.

$C_5H_{10}Cl_2N_2O$  185.05



## Carmustine for Injection

### DEFINITION

Carmustine for Injection is a sterile lyophilized preparation of carmustine. It contains NLT 90.0% and NMT 110.0% of the labeled amount of carmustine ( $C_5H_9Cl_2N_3O_2$ ).

**[CAUTION—**Use appropriate surgical gloves, arm covers, and a dust mask. Perform all work under a fume hood approved for testing cytotoxic agents when possible.]

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197F)

**Sample:** Melt a small portion of the sample in a suitable container in a controlled water bath or oven, and set the temperature between 33° and 40°.

**Standard:** A similar preparation of USP Carmustine RS

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

[NOTE—Prepare solutions in low-actinic glassware, and keep them refrigerated until use.]

**Mobile phase:** See the gradient table below.

Time (min)	Water (%)	Acetonitrile (%)
0	90	10
2.5	90	10
7	40	60
8.5	90	10
10.5	90	10

**Diluent:** Acetonitrile and water (1:3)

**Standard stock solution:** 2.0 mg/mL of USP Carmustine RS in acetonitrile

**Standard solution:** 0.2 mg/mL of USP Carmustine RS in *Diluent*, from *Standard stock solution*

**Impurity standard stock solution:** 0.1 mg/mL of USP Carmustine Related Compound A RS in acetonitrile

**System suitability solution:** 0.2 mg/mL of USP Carmustine RS and 0.002 mg/mL of USP Carmustine Related Compound A RS in *Diluent*, from the *Standard stock solution* and *Impurity standard stock solution*, respectively

**Sample stock solution:** 2.0 mg/mL of carmustine in acetonitrile, from Carmustine for Injection. [NOTE—Allow test vials to warm to room temperature in a desiccator for 1 h.]

**Sample solution:** 0.2 mg/mL of carmustine in *Diluent*, from the *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 200 nm

**Refrigerated autosampler temperature:** 5°

**Column:** 4.6-mm × 7.5-cm; 3-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 20 μL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for carmustine related compound A and carmustine are 0.5 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between carmustine related compound A and carmustine

**Tailing factor:** NMT 1.5 for the carmustine related compound A and carmustine peaks

**Relative standard deviation:** NMT 2.0% for the carmustine related compound A and carmustine peaks

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carmustine ( $C_5H_9Cl_2N_3O_2$ ) in the portion of Carmustine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of the *Sample solution*

$r_S$  = peak area of the *Standard solution*

$C_S$  = concentration of carmustine in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carmustine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

### IMPURITIES

#### Organic Impurities

- **PROCEDURE: LIMIT OF CARMUSTINE RELATED COMPOUND A**

*Diluent*, *Impurity standard stock solution*, *System suitability solution*, *Sample solution*, *Chromatographic system*, and *System suitability*: Proceed as directed in the *Assay*.

**Standard solution:** 0.002 mg/mL of USP Carmustine Related Compound A RS in *Diluent*, from the *Impurity standard stock solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carmustine related compound A in the portion of Carmustine for Injection taken:

$$\text{Result} = (r_U/r_S) \times [100 \times C_S/(C_U \times A)] \times 100$$

$r_U$  = peak response of carmustine related compound A from the *Sample solution*

$r_S$  = peak response of carmustine related compound A from the *Standard solution*

$C_S$  = concentration of carmustine related compound A in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carmustine in the *Sample solution* (mg/mL)

$A$  = assay of Carmustine for Injection, as a percentage

#### Acceptance criteria

**Carmustine related compound A:** NMT 1.0%

### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.95 USP Endotoxin Unit/mg of carmustine
- **STERILITY TESTS** (71): Meets the requirements
- **PH** (791): Between 4.0 and 6.8 in a constituted solution prepared as directed in the labeling
- **WATER DETERMINATION, Method I** (921): NMT 1.0%

### ADDITIONAL REQUIREMENTS

#### Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 11-May-2017) at a temperature between 2° and 8°.
- **LABELING:** It meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*.
- **CONSTITUTED SOLUTION:** At time of use, it meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.
- **USP REFERENCE STANDARDS** (11)  
USP Carmustine RS  
Urea, *N,N'*-bis(2-chloroethyl)-*N*-nitroso;



1,3-Bis(2-chloroethyl)-1-nitrosourea.

 $C_5H_9Cl_2N_3O_2$  214.05

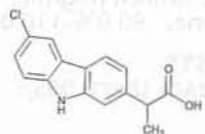
USP Carmustine Related Compound A RS

1,3-Bis(2-chloroethyl) urea.

 $C_5H_{10}Cl_2N_2O$  185.05

USP Endotoxin RS

## Carprofen

 $C_{15}H_{12}ClNO_2$  273.71

9H-Carbazole-2-acetic acid, 6-chloro-α-methyl-, (±)-.

(±)-6-Chloro-α-methylcarbazole-2-acetic acid

[53716-49-7].

» Carprofen contains not less than 98.0 percent and not more than 102.0 percent of  $C_{15}H_{12}ClNO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Label it to indicate that it is intended for veterinary use only.

### USP Reference standards (11)—

USP Carprofen RS

USP Carprofen Related Compound A RS

Carbazole.

 $C_{12}H_9N$  167.21

### Identification—

A: Infrared Absorption (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Loss on drying** (731)—Dry it at 105° for 2 hours; it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

### Delete the following:

• **Heavy metals, Method II** (231): 0.002%. (Official 1-Jan-2018)

### Limit of acetone and methylene chloride—

**Standard solution**—Transfer about 5.0 g of acetone and 0.6 g of methylene chloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *N,N*-dimethylacetamide to volume, and mix. Pipet 1 mL of this solution into a 100-mL volumetric flask, dissolve in and dilute with *N,N*-dimethylacetamide to volume, and mix.

**Test solution**—Transfer about 500 mg of Carprofen, accurately weighed, to a 5-mL volumetric flask, dissolve in and dilute with *N,N*-dimethylacetamide to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m capillary column coated with 3.0-μm G43 stationary phase. The carrier gas is nitrogen, flowing at a rate of about 4.9 mL per minute. The split flow ratio is about 10:1. Initially the column temperature is maintained at 80° for 4 minutes, then is increased at a rate of 30° per minute to a temperature of 190°, and maintained at 190° for at least 3 minutes. The injection port temperature is maintained at 210°, and the detector temperature is

maintained at 220°. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: acetone elutes before methylene chloride; the resolution, *R*, between them is not less than 1.5; and the relative standard deviation for replicate injections, determined from the peak responses of acetone, is not more than 10.0%.

**Procedure**—Separately inject equal volumes (about 1 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all the peak responses. Calculate the percentage of each residual solvent in the portion of Carprofen taken by the formula:

$$0.5(C_s / W)(r_u / r_s)$$

in which  $C_s$  is the concentration, in μg per mL, of the individual residual solvent in the *Standard solution*;  $W$  is the weight, in mg, of Carprofen taken to prepare the *Test solution*;  $r_u$  is the peak response of the individual residual solvent in the *Test solution*; and  $r_s$  is the peak response of the individual residual solvent in the *Standard solution*: not more than 5000 ppm of acetone is found; and not more than 600 ppm of methylene chloride is found.

### Related compounds—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay*.

**Test solution**—Use the *Assay preparation*.

**Procedure**—Inject about 10 μL of the *Test solution* into the chromatograph, record the chromatogram, and measure the responses for all the peaks. Calculate the percentage of each related compound in the portion of Carprofen taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the response of each individual peak other than the major peak of carprofen; and  $r_s$  is the sum of the peak responses: not more than 0.5% of each individual known related compound is found (see the relative retention times of these compounds in the table below); not more than 0.1% of each individual unknown related compound is found; and not more than 1.0% of total related compounds is found.

Known Related Compound	Approximate Relative Retention Time
Carprofen related compound A (carbazole)	0.9
2-[1,1-Dimethoxy-2-hydroxypropyl]-6-chlorocarbazole	1.3
2-[2-Chloropropionyl]-6-chloro-9-acetylcarbazole	3.3

### Assay—

**Mobile phase**—Prepare a mixture of acetonitrile, water, methanol, and glacial acetic acid (40:35:25:0.2).

**Carprofen related compound A solution**—[NOTE—Use low-actinic glassware.] Prepare a solution of USP Carprofen Related Compound A RS, accurately weighed, in *Mobile phase*, containing about 16 μg per mL, sonicating if necessary.

**Standard preparation**—[NOTE—Use low-actinic glassware.] Prepare a solution of USP Carprofen RS, accurately weighed, in *Mobile phase*, containing about 160 μg per mL, sonicating if necessary.

**System suitability solution**—[NOTE—Use low-actinic glassware.] Transfer 10 mL of *Carprofen related compound A solution* and 10 mL of *Standard preparation* into a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of Carprofen in *Mobile phase*, and dilute quantitatively, and stepwise if necessary,



to obtain a solution having a known concentration of about 160 µg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 239-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R_s$ , between carprofen and carprofen related compound A is not less than 2.0; the column efficiency for the carprofen peak is not less than 5000 theoretical plates; the tailing factor for the carprofen peak is not more than 2.0; and the relative standard deviation for replicate injections of carprofen is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all the peaks. Calculate the percentage of  $C_{15}H_{12}ClNO_2$  in the portion of Carprofen taken by the formula:

$$100P(C_s / C_u)(R_u / R_s)$$

in which  $P$  is the purity, in µg per mg, of USP Carprofen RS;  $C_s$  and  $C_u$  are the concentrations, in µg per mL, of the *Standard preparation* and the *Assay preparation*, respectively; and  $R_u$  and  $R_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Carprofen Tablets

» Carprofen Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of carprofen ( $C_{15}H_{12}ClNO_2$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Carprofen RS

**Identification**—

**A: Infrared Absorption** (197K)—

**Reference specimen**—Mix about 2 mg of USP Carprofen RS with 200 mg of potassium bromide, and grind thoroughly for 10 to 15 minutes. Compress the mixture into a clear pellet. Record the IR spectrum of the pellet immediately after preparation.

**Test specimen**—Grind into powder not fewer than 4 Tablets. Transfer the powder, equivalent to about 100 mg of carprofen, to a 125-mL separatory funnel. Add 30 mL of water and 3 drops of hydrochloric acid, and shake for about 5 minutes. Add about 30 mL of methylene chloride, and shake for another 5 minutes. Allow the phases to separate. Carefully drain and collect the lower methylene chloride layer through anhydrous sodium sulfate that is placed on a cotton pledget into a suitable container. Evaporate the methylene chloride on a steam bath with the aid of a stream of nitrogen to dryness. Dry the residue in vacuum at 60° for about 30 minutes. Mix about 2 mg of the dried residue with 200 mg of potassium bromide, and grind thoroughly for 10 to 15 minutes. Compress the mixture into a clear pellet. Record the IR spectrum of the carprofen sample pellet immediately after preparation.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—[NOTE—Use low-actinic volumetric flasks, dissolution vessels, and evaporation covers.]

**Medium:** 0.05 M phosphate buffer, pH 7.5 (prepared by dissolving 6.8 g of monobasic potassium phosphate in

600 mL of water, mixing, adding 18 mL of 2 N sodium hydroxide, mixing, diluting with water to 1000 mL, and adjusting with 0.2 N sodium hydroxide or 0.2 N hydrochloric acid to a pH of  $7.50 \pm 0.05$ ); 900 mL, degassed.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

Determine the amount of  $C_{15}H_{12}ClNO_2$  dissolved by employing the following method.

**Standard solution**—

FOR TABLETS LABELED TO CONTAIN 25 MG—Transfer about 25 mg of USP Carprofen RS, accurately weighed, to a 900-mL volumetric flask. Slowly add 10 mL of methanol. Dilute with *Medium* to volume, and mix.

FOR TABLETS LABELED TO CONTAIN 75 MG—Transfer about 75 mg of USP Carprofen RS, accurately weighed, to a 900-mL volumetric flask. Slowly add 30 mL of methanol. Dilute with *Medium* to volume, and mix.

FOR TABLETS LABELED TO CONTAIN 100 MG—Transfer about 100 mg of USP Carprofen RS, accurately weighed, to a 900-mL volumetric flask. Slowly add 40 mL of methanol. Dilute with *Medium* to volume, and mix.

**Test solution**—Pass a portion of the solution under test through a suitable 0.45-µm filter.

**System suitability solution**—Determine the absorbance of the *Standard solution*, as directed for *Procedure*, five times: the relative standard deviation is not more than 2.0%.

**Procedure**—Determine the amount of  $C_{15}H_{12}ClNO_2$  dissolved by measuring the absorbance of the *Test solution* in comparison with the appropriate *Standard solution* at the wavelength of maximum absorbance at about 300 nm, using a 0.5-cm cell for Tablets labeled to contain 25 mg, a 0.2-cm cell for Tablets labeled to contain 75 mg, and a 0.1-cm cell for Tablets labeled to contain 100 mg. Use *Medium* as the blank. Calculate the percentage of  $C_{15}H_{12}ClNO_2$  dissolved by the formula:

$$\frac{A_u \times W_s \times 100}{A_s \times LC}$$

in which  $A_u$  and  $A_s$  are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively;  $W_s$  is the weight, in mg, of USP Carprofen RS used to prepare the *Standard solution*; 100 is the conversion factor to percentage; and  $LC$  is the Tablet label claim, in mg.

**Tolerances**—Not less than 80% ( $Q$ ) of the labeled amount of  $C_{15}H_{12}ClNO_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements for *Content Uniformity*.

PROCEDURE FOR CONTENT UNIFORMITY—[NOTE—Use low-actinic glassware.]

**Mobile phase and Chromatographic system**—Prepare as directed in the *Assay*.

**Standard solution**—Prepare as directed for the *Standard preparation* in the *Assay*.

**Test solution**—Transfer 10 Tablets individually to 10 separate volumetric flasks of a suitable calibrated volume such that an interim concentration of 0.5 mg per mL of *Mobile phase* can be prepared. To each flask, add *Mobile phase* to 80% of the calibrated volume, sonicate for 10 minutes, then stir for 10 minutes. Sonicate again for 10 minutes, and stir for another 10 minutes or until the Tablets are completely disintegrated. Cool to room temperature, dilute with *Mobile phase* to volume to obtain an interim concentration of 0.5 mg of carprofen per mL, and mix. Quantitatively transfer 5.0 mL of the individual solutions to 10 separate 50.0-mL volumetric flasks, dilute with *Mobile phase* to volume, and mix. Pass the solution through a polyvinylidene fluoride (PVDF) filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate. The final concentration is about 0.05 mg of carprofen per mL.



**Procedure**—Proceed as directed for *Procedure* in the Assay. Calculate the percentage of the labeled content of  $C_{15}H_{12}ClNO_2$  in the portion of Tablets taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which the terms are as defined therein.

#### Chromatographic purity—

**Mobile phase**—Proceed as directed in the Assay.

**Standard solution**—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Carprofen RS in *Mobile phase* to obtain a solution having a known concentration of 0.05 µg of carprofen per mL.

**Sensitivity solution**—[NOTE—Use low-actinic glassware.] Quantitatively dilute the *Standard solution* with *Mobile phase* to obtain a solution containing about 0.005 µg of carprofen per mL.

**Test solution**—Use the Assay preparation.

**Blank solution**—Transfer an accurately weighed portion of the Tablet base, equivalent to the weight of 1 Tablet, to a volumetric flask of the same calibrated volume as that used to prepare the *Test solution*. To each flask add *Mobile phase* to 80% of the calibrated volume. Sonicate for 10 minutes, then stir for 10 minutes. Sonicate again for 10 minutes, and stir for another 10 minutes. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Quantitatively transfer 5.0 mL of the solution to a 50.0-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass the solution through a PVDF filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L7. The flow rate is about 1.0 mL per minute. Wash the column after each series of analyses with a mixture of acetonitrile and water (20:80) for 30 minutes; gradually change the composition of acetonitrile and water to 80:20 over 10 minutes; continue to wash at 80:20 for 30 minutes; gradually change the composition to 50:50 over 10 minutes; and continue to wash at 50:50 for another 30 minutes. Chromatograph the *Standard solution*, the *Sensitivity solution*, and the *Test solution*, and record the peak responses as directed for *Procedure*: for the *Standard solution*, the column efficiency is not less than 4000 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%; for the *Sensitivity solution*, the carprofen peak should be defined and integratable; for the *Test solution*, the resolution,  $R$ , between carprofen and the nearest impurity peak is not less than 2.0. After every six injections of any solution, inject a *Standard solution* in duplicate. The ratio of the average response of the duplicate injections to that obtained from the initial five replicate injections is 0.95 to 1.05.

**Procedure**—Inject a volume (about 50 µL) of the *Standard solution*, the *Test solution*, and the *Blank solution* into the chromatograph, record the chromatograms, and measure all the peak areas. Calculate the percentage of carprofen-related compounds in the portion of Tablets taken by the formula:

$$0.1(C_s / C_u)(r_i / r_s)$$

in which  $C_s$  is the concentration, in µg per mL, of carprofen in the *Standard solution*;  $C_u$  is the concentration, in mg per mL, of carprofen in the *Test solution*;  $r_i$  is the peak area of any peak other than carprofen obtained from the *Test solu-*

*tion*; and  $r_s$  is the peak area of carprofen obtained from the *Standard solution*: not more than 0.5% of any single impurity is found; and the sum of all impurities is not more than 2.0%. Disregard any peak also observed in the *Blank solution*.

#### Assay—

**Mobile phase**—Mix 500 mL of acetonitrile, 500 mL of water, and 1 mL of phosphoric acid. Degas before using. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—[NOTE—Use low-actinic glassware.] Dissolve an accurately weighed quantity of USP Carprofen RS in *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

**Assay preparation**—[NOTE—Use low-actinic glassware.] Accurately weigh 20 Tablets, and calculate the average Tablet weight. Grind the Tablets into uniform powder. Transfer three accurately weighed portions of the powder, each equivalent to the weight of one Tablet, into three volumetric flasks of a suitable calibrated volume such that an interim concentration of 0.5 mg per mL of *Mobile phase* can be prepared. To each flask add *Mobile phase* to 80% of the calibrated volume, sonicate for 10 minutes, then stir for 10 minutes. Sonicate again for 10 minutes, and stir for another 10 minutes. Cool to room temperature, dilute with *Mobile phase* to volume to obtain an interim concentration of 0.5 mg of carprofen per mL, and mix. Quantitatively transfer 5.0 mL of the solution to a 50.0-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Pass the solution through a PVDF filter having a 0.45-µm or finer porosity, discarding the first 5 mL of the filtrate. The final concentration is about 0.05 mg of carprofen per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Wash the column after each series of analyses with a mixture of acetonitrile and water (20:80) for 30 minutes; gradually change the composition of acetonitrile and water to 80:20 over 10 minutes; continue to wash at 80:20 for 30 minutes; gradually change the composition to 50:50 over 10 minutes; and continue to wash at 50:50 for another 30 minutes. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the column efficiency for carprofen is not less than 4000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for five replicate injections is not more than 2.0%. Inject the *Standard preparation* in duplicate after every 12 injections or fewer of any other solution. The ratio of the average area of the duplicate injections to that obtained from the initial five replicate injections is 0.95 to 1.05.

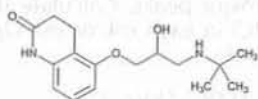
**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of the labeled content of carprofen ( $C_{15}H_{12}ClNO_2$ ) in the portion of Tablets taken by the formula:

$$100(C_s / C_u)(r_u / r_s)$$

in which  $C_s$  and  $C_u$  are the concentrations, in mg per mL, of USP Carprofen RS in the *Standard preparation* and carprofen in the *Assay preparation*, respectively; and  $r_u$  and  $r_s$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Carteolol Hydrochloride



$C_{16}H_{24}N_2O_3 \cdot HCl$  328.83  
 2(1*H*)-Quinolinone, 5-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-3,4-dihydro-, monohydrochloride;  
 5-[3-(*tert*-Butylamino)-2-hydroxypropoxy]-3,4-dihydrocarbo-2-styryl monohydrochloride [51781-21-6].

### DEFINITION

Carteolol Hydrochloride contains NLT 98.0% and NMT 101.5% of carteolol hydrochloride ( $C_{16}H_{24}N_2O_3 \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)  
 Sample solution: 10 µg/mL  
 Medium: Water  
 Acceptance criteria: Meets the requirements
- **C. CHLORIDE AND SULFATE, Chloride** (191)  
 Sample solution: 20 mg/mL  
 Acceptance criteria: Meets the requirements

### ASSAY

#### • PROCEDURE

**Buffer:** 0.67 g/L of dibasic sodium phosphate prepared as follows. Dissolve 1.34 g of dibasic sodium phosphate in 1900 mL of water, adjust with 1 M phosphoric acid to a pH of  $6.0 \pm 0.05$ , and dilute with water to volume.  
**Mobile phase:** Acetonitrile and Buffer (250:750)

[NOTE—Increasing the proportion of pH 6.0 buffer increases resolution.]

**Standard stock solution:** 1 mg/mL of USP Carteolol Hydrochloride RS in water

**Standard solution:** 0.1 mg/mL of USP Carteolol Hydrochloride RS from *Standard stock solution* prepared as follows. Transfer 10 mL of *Standard stock solution* to a 100-mL volumetric flask containing 5 mL of acetonitrile, and dilute with water to volume.

**System suitability stock solution:** Dissolve 50 mg of *p*-acetotoluidide in a 100-mL volumetric flask in 50 mL of acetonitrile, and dilute with water to volume.

**System suitability solution:** 0.05 mg/mL of *p*-acetotoluidide and 0.1 mg/mL of USP Carteolol Hydrochloride RS in water from *System suitability stock solution* and *Standard stock solution*

**Sample stock solution:** 1 mg/mL of Carteolol Hydrochloride in water

**Sample solution:** 0.1 mg/mL of Carteolol Hydrochloride in water prepared as follows. Transfer 10.0 mL of *Sample stock solution* to a 100-mL volumetric flask containing 5 mL of acetonitrile, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 252 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for carteolol and *p*-acetotoluidide are 0.8 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 3 between carteolol and *p*-acetotoluidide, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carteolol hydrochloride ( $C_{16}H_{24}N_2O_3 \cdot HCl$ ) in the portion of Carteolol Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Carteolol Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carteolol Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–101.5% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%
- **ARSENIC, Method II** (211): NMT 3 ppm

### Delete the following:

- **HEAVY METALS, Method I** (231): NMT 20 ppm (Official 1-Jan-2018)

### • ORGANIC IMPURITIES

**Standard solution A:** 0.5 mg/mL of USP Carteolol Hydrochloride RS in methanol

**Standard solution B:** Dilute 5.0 mL of *Standard solution A* with methanol to 50 mL.

**Standard solution C:** Dilute 5.0 mL of *Standard solution B* with methanol to 10 mL.

**Sample solution:** Transfer 250 mg of Carteolol Hydrochloride to a 10-mL volumetric flask, and dissolve in methanol, using heat or sonication if necessary to achieve dissolution. Dilute with methanol to volume.

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10 µL

**Developing solvent system:** Chloroform, methanol, and ammonium hydroxide (50:20:1)

### Analysis

**Samples:** *Standard solutions A, B, and C* and *Sample solution*

Line a chromatographic chamber with filter paper, and saturate the paper with the *Developing solvent system*. Allow the spots on the plate to dry, place the plate in the chamber, and develop until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the chamber, and allow to air-dry. Examine the plate under short-wavelength UV light.

### Acceptance criteria

The  $R_f$  value of the principal spot from the *Sample solution* corresponds to that from *Standard solution A*.

**Individual impurities:** Compare the sizes and intensities of any spots other than the principal spot from the *Sample solution* with those of the principal spots from the *Standard solutions*: no spot exceeds in size or intensity the principal spot from *Standard solution B*, NMT 0.2%.

**Total impurities:** The sum of all the impurity spots is NMT 0.5%.



**SPECIFIC TESTS**

- **pH (791)**  
Sample solution: 10 mg/mL  
Acceptance criteria: 5.0–6.0
- **LOSS ON DRYING (731)**  
Analysis: Dry a sample at 105° for 3 h.  
Acceptance criteria: NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
USP Carteolol Hydrochloride RS

**Carteolol Hydrochloride Ophthalmic Solution**

» Carteolol Hydrochloride Ophthalmic Solution is a sterile, aqueous, isotonic solution of Carteolol Hydrochloride. It contains a suitable antimicrobial preservative. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{24}N_2O_3 \cdot HCl$ .

**Packaging and storage—**Preserve in tight containers.

**USP Reference standards (11)—**

USP Carteolol Hydrochloride RS

**Identification—**

**A:** Prepare a test solution by diluting a suitable volume of Ophthalmic Solution with water, if necessary, to obtain a solution containing about 1 mg of carteolol hydrochloride per mL. Separately apply 10  $\mu$ L of the test solution and 10  $\mu$ L of a Standard solution of USP Carteolol Hydrochloride RS in water containing about 1 mg per mL to the starting line of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry. Line a chromatographic chamber with filter paper, and saturate the paper with a solvent system consisting of a mixture of chloroform, methanol, and ammonium hydroxide (50:20:1). Place the plate in the chamber, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow to air-dry. Examine the plate under short-wave-length UV light: the  $R_f$  value of the principal spot in the chromatogram obtained from the test solution corresponds to that in the chromatogram obtained from the Standard solution.

**B:** The retention time of the carteolol peak in the chromatogram of the *Assay preparation* obtained as directed in the *Assay* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Sterility Tests (71)—**It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH (791):** between 6.0 and 8.0.

**Assay—**

pH 6.0 buffer, Mobile phase, Diluent, Standard preparation, Resolution solution, and Chromatographic system—Proceed as directed in the *Assay* under *Carteolol Hydrochloride*.

**Assay preparation—**Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of carteolol hydrochloride, to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass a portion of this solution through a filter having a porosity of 0.5  $\mu$ m or finer, discarding the first 2 mL of the filtrate, and use the filtrate as the *Assay preparation*.

**Procedure—**Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $C_{16}H_{24}N_2O_3 \cdot HCl$  in each mL of the Ophthalmic Solution taken by the formula:

$$100(C/V)(r_u/r_s)$$

in which C is the concentration, in mg per mL, of USP Carteolol Hydrochloride RS in the *Standard preparation*; V is the volume, in mL, of Ophthalmic Solution taken; and  $r_u$  and  $r_s$  are the carteolol peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Carteolol Hydrochloride Tablets****DEFINITION**

Carteolol Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carteolol hydrochloride ( $C_{16}H_{24}N_2O_3 \cdot HCl$ ).

**IDENTIFICATION**

- **A.** The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 0.67 g/L of dibasic sodium phosphate prepared as follows. Dissolve 1.34 g of dibasic sodium phosphate in 1900 mL of water, adjust with 1 M phosphoric acid to a pH of  $6.0 \pm 0.05$ , and dilute with water to volume.

**Mobile phase:** Acetonitrile and *Buffer* (250:750)

[NOTE—Increasing the proportion of buffer increases resolution.]

**Diluent:** Methanol and *Buffer* (50:50)

**Standard stock solution:** 1 mg/mL of USP Carteolol Hydrochloride RS in water

**Standard solution:** 0.1 mg/mL of USP Carteolol Hydrochloride RS from *Standard stock solution* prepared as follows. Transfer 10 mL of *Standard stock solution* to a 100-mL volumetric flask containing 5 mL of acetonitrile, and dilute with water to volume.

**System suitability stock solution:** Dissolve 50 mg of *p*-acetotoluidide in a 100-mL volumetric flask in 50 mL of acetonitrile, and dilute with water to volume.

**System suitability solution:** 0.05 mg/mL of *p*-acetotoluidide and 0.1 mg/mL of USP Carteolol Hydrochloride RS in water from *System suitability stock solution* and *Standard stock solution*

**Sample solution:** Nominally 0.1 mg/mL of Carteolol Hydrochloride in *Diluent* prepared as follows. Transfer an amount equivalent to 10 mg of carteolol hydrochloride from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add 50 mL of *Diluent*, and shake by mechanical means for 1 h. Add 5 mL of acetonitrile, and dilute with *Diluent* to volume. Pass a portion of this solution through a suitable filter of 0.5- $\mu$ m or finer pore size, discarding the first 2 mL of filtrate. Use the clear filtrate as the *Sample solution*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 252 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for carteolol and *p*-acetotoluidide are 0.8 and 1.0, respectively.]



**Suitability requirements**

**Resolution:** NLT 3 between carteolol and *p*-acetotoluidide, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carteolol hydrochloride ( $C_{16}H_{24}N_2O_3 \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of carteolol hydrochloride from the *Sample solution*

$r_S$  = peak area of carteolol hydrochloride from the *Standard solution*

$C_S$  = concentration of USP Carteolol Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carteolol hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION (711)**

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Buffer:** Dissolve 2.0 g of monobasic potassium phosphate in water to make 1 L of solution.

**Mobile phase:** Acetonitrile and *Buffer* (400:600)

**Standard solution:** 1.1 L µg/mL of USP Carteolol Hydrochloride RS, where *L* is the labeled amount, in mg, of carteolol hydrochloride per Tablet

**Sample solution:** Pass a portion of the solution under test through a filter of 1-µm or finer pore size, discarding the first 2 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 252 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 15 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of carteolol hydrochloride ( $C_{16}H_{24}N_2O_3 \cdot HCl$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response of the *Sample solution*

$r_S$  = peak response of the *Standard solution*

$C_S$  = concentration of USP Carteolol Hydrochloride RS in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of medium, 900 mL

**Tolerances:** NLT 80% (*Q*) of the labeled amount of carteolol hydrochloride ( $C_{16}H_{24}N_2O_3 \cdot HCl$ )

**• UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**IMPURITIES****• LIMIT OF DEHYDROCARTEOLOL HYDROCHLORIDE**

**Buffer, Mobile phase, and Diluent:** Proceed as directed in the *Assay*.

**Standard solution:** 1 µg/mL of USP Dehydrocarteolol Hydrochloride RS in *Diluent*

**Sample solution:** Transfer an amount nominally equivalent to 10 mg of carteolol hydrochloride from NLT 20 finely powdered Tablets to a 100-mL volumetric flask,

add 50 mL of *Diluent*, and shake by mechanical means for 1 h. Dilute with *Diluent* to volume. Pass a portion of this solution through a filter of 0.5-µm or finer pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Fluorometric, with excitation at 300 nm and a 418-nm emission filter

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 5%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of dehydrocarteolol hydrochloride in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of dehydrocarteolol from the *Sample solution*

$r_S$  = peak response of dehydrocarteolol from the *Standard solution*

$C_S$  = concentration of USP Dehydrocarteolol Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of carteolol hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 1.0%

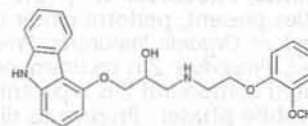
**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight containers.**• USP REFERENCE STANDARDS (11)**

USP Carteolol Hydrochloride RS

USP Dehydrocarteolol Hydrochloride RS

5-(3-*tert*-Butylamino-2-hydroxy)-propoxycarbostyryl hydrochloride.

$C_{16}H_{22}N_2O_3 \cdot HCl$  326.82

**Carvedilol**

$C_{24}H_{26}N_2O_4$

2-Propanol, 1-(9*H*-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]-, (±)-; (±)-1-(Carbazol-4-yloxy)-3-[[2-(*o*-methoxyphenoxy)ethyl]amino]-2-propanol [72956-09-3].

406.47

**DEFINITION**

Carvedilol contains NLT 98.0% and NMT 102.0% of  $C_{24}H_{26}N_2O_4$ , calculated on the dried basis.

**IDENTIFICATION****• A. INFRARED ABSORPTION (197K)**

**B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Buffer:** 2.72 g/L of monobasic potassium phosphate. Adjust with dilute phosphoric acid to a pH of 2.0.



**Mobile phase:** Acetonitrile and Buffer (31:69)

**System suitability solution:** 0.05 mg/mL each of USP Carvedilol RS and USP Carvedilol Related Compound A RS in *Mobile phase*

**Standard solution:** 0.04 mg/mL of USP Carvedilol RS in *Mobile phase*

**Sample solution:** 0.04 mg/mL of Carvedilol in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L7

**Column temperature:** 55°

**Flow rate:** 1 mL/min

**Run time:** 60 min

**Injection size:** 10 μL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 4.0 between carvedilol and carvedilol related compound A

**Tailing factor:** NMT 1.5 for the carvedilol peak

**Relative standard deviation:** NMT 2%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carvedilol (C<sub>24</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of carvedilol from the *Sample solution*

$r_S$  = peak response of carvedilol from the *Standard solution*

$C_S$  = concentration of carvedilol in the *Standard solution* (mg/mL)

$C_U$  = concentration of Carvedilol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1% from 1 g

#### Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 10 ppm (Official 1, Jan-2018)

- **ORGANIC IMPURITIES, PROCEDURE 1:** [NOTE—On the basis of the impurities present, perform either *Organic Impurities, Procedure 1* or *Organic Impurities, Procedure 2*. *Organic Impurities, Procedure 2* is recommended when carvedilol related compound F is a potential impurity.] **Buffer and Mobile phase:** Prepare as directed in the *Assay*.

**System suitability solution:** 0.05 mg/mL each of USP Carvedilol RS and USP Carvedilol Related Compound C RS in *Mobile phase*

**Standard solution:** 1 μg/mL each of USP Carvedilol RS, USP Carvedilol Related Compound A RS, USP Carvedilol Related Compound B RS, USP Carvedilol Related Compound D RS, and USP Carvedilol Related Compound E RS, and 0.2 μg/mL of USP Carvedilol Related Compound C RS in *Mobile phase*

**Sample solution:** 1 mg/mL of Carvedilol in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Dual wavelength, UV 220 and 240 nm. Use 220 nm for quantitating carvedilol related compound E, and use 240 nm for carvedilol and all other related compounds.

**Column:** 4.6-mm × 15-cm; 5-μm packing L7

**Column temperature:** 55°

**Flow rate:** 1 mL/min

**Injection size:** 20 μL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 17 between carvedilol and carvedilol related compound C

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of carvedilol related compound A, carvedilol related compound B, carvedilol related compound C, carvedilol related compound D, carvedilol related compound E, and any other individual impurity in the portion of Carvedilol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the corresponding related compound or any other impurity from the *Sample solution*

$r_S$  = peak response of the corresponding related compound from the *Standard solution*. To calculate the percentage of any other individual impurity use the peak response of carvedilol.

$C_S$  = concentration of the corresponding related compound in the *Standard solution* (mg/mL). To calculate the percentage of any other impurities for  $C_S$ , use the concentration of USP Carvedilol RS.

$C_U$  = concentration of Carvedilol in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carvedilol related compound E <sup>a</sup>	0.35	0.1
Carvedilol related compound A <sup>b</sup>	0.52	0.1
Carvedilol bisalkylpyrocatechol derivative (if present) <sup>c</sup>	0.70	0.15
Carvedilol	1.0	—
Carvedilol related compound C <sup>d</sup>	3.6	0.02
Carvedilol related compound D <sup>e</sup>	5.0	0.1
Carvedilol related compound B <sup>f</sup>	8.5	0.1
Any other individual impurity	—	0.10
Total impurities	—	0.5 <sup>g</sup>

<sup>a</sup> 2-(2-Methoxyphenoxy)ethyl amine.

<sup>b</sup> 1-(4-(2-Hydroxy-3-(2-(2-methoxyphenoxy)ethylamino)propoxy)-9H-carbazol-9-yl)-3-(2-(2-methoxyphenoxy)ethylamino) propan-2-ol.

<sup>c</sup> 3,3'-(2,2'-[1,2-Phenylenebis(oxy)]bis(ethane-2,1-diyl))bis(azanediy)bis(1-(9H-carbazol-4-yloxy)propan-2-ol).

<sup>d</sup> 1-(9H-Carbazol-4-yloxy)-3-(benzyl(2-(2-methoxyphenoxy)ethyl)-amino)propan-2-ol.

<sup>e</sup> 4-(Oxiran-2-ylmethoxy)-9H-carbazole.

<sup>f</sup> 3,3'-(2-(2-Methoxyphenoxy)ethylazanediy)bis(1-(9H-carbazol-4-yloxy)propan-2-ol).

<sup>g</sup> Disregard any impurity less than 0.01%.

- **ORGANIC IMPURITIES, PROCEDURE 2**

**Solution A:** Acetonitrile and trifluoroacetic acid (100:0.1)

**Solution B:** Trifluoroacetic acid and water (0.1:100)

**Diluent:** Acetonitrile, trifluoroacetic acid, and water (22:0.1:78)

**Mobile phase:** See *Table 2*.



Table 2

Time (min)	Solution A (%)	Solution B (%)
0	22	78
20	22	78
33	38	62
45	38	62
55	55	45
65	55	45
68	22	78
80	22	78

**System suitability solution:** 1.0 mg/mL of USP Carvedilol System Suitability Mixture RS in *Diluent*  
**Sample solution:** 1 mg/mL of Carvedilol in *Diluent*  
**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L68

**Column temperature:** 30°

**Flow rate:** 1.4 mL/min

**Injection size:** 20 μL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 1.8 between carvedilol and carvedilol related compound F

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Carvedilol taken:

$$\text{Result} = (r_u/r_r) \times 100$$

$r_u$  = peak response for each impurity in the *Sample solution*

$r_r$  = sum of all the peak responses in the *Sample solution*

**Acceptance criteria:** See Table 3.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Carvedilol related compound A <sup>a</sup>	0.7	0.1
Carvedilol	1.0	—
Carvedilol related compound F <sup>b</sup>	1.2	0.1 <sup>c</sup>
<i>N</i> -Isopropylcarvedilol <sup>d</sup>	1.6	0.1
Carvedilol related compound C <sup>e</sup>	1.8	0.02
Carvedilol related compound B <sup>f</sup>	2.1	0.1
Biscarbazole <sup>g</sup>	3	0.1

<sup>a</sup> 1-(4-(2-Hydroxy-3-(2-(2-methoxyphenoxy)ethylamino)propoxy)-9H-carbazol-9-yl)-3-(2-(2-methoxyphenoxy)ethylamino) propan-2-ol.

<sup>b</sup> 1-(2-(2-Methoxyphenoxy)ethylamino)-3-(6,7,8,9-tetrahydro-5H-carbazol-4-yloxy)propan-2-ol.

<sup>c</sup> This impurity is quantitated using the procedure under *Organic Impurities, Procedure 3: Carvedilol Related Compound F*.

<sup>d</sup> 1-(*H*-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]*N*-isopropylamino]-2-propanol.

<sup>e</sup> 1-(9H-Carbazol-4-yloxy)-3-(benzyl(2-(2-methoxyphenoxy)ethyl)amino)-propan-2-ol.

<sup>f</sup> 3,3'-(2-(2-Methoxyphenoxy)ethylazanediyl)bis(1-(9H-carbazol-4-yloxy)propan-2-ol).

<sup>g</sup> 1,3-Bis-(9H-carbazol-4-yloxy)-2-propanol.

Table 3 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any other individual impurity	—	0.1
Total impurities	—	0.5

<sup>a</sup> 1-(4-(2-Hydroxy-3-(2-(2-methoxyphenoxy)ethylamino)propoxy)-9H-carbazol-9-yl)-3-(2-(2-methoxyphenoxy)ethylamino) propan-2-ol.

<sup>b</sup> 1-(2-(2-Methoxyphenoxy)ethylamino)-3-(6,7,8,9-tetrahydro-5H-carbazol-4-yloxy)propan-2-ol.

<sup>c</sup> This impurity is quantitated using the procedure under *Organic Impurities, Procedure 3: Carvedilol Related Compound F*.

<sup>d</sup> 1-(*H*-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]*N*-isopropylamino]-2-propanol.

<sup>e</sup> 1-(9H-Carbazol-4-yloxy)-3-(benzyl(2-(2-methoxyphenoxy)ethyl)amino)-propan-2-ol.

<sup>f</sup> 3,3'-(2-(2-Methoxyphenoxy)ethylazanediyl)bis(1-(9H-carbazol-4-yloxy)propan-2-ol).

<sup>g</sup> 1,3-Bis-(9H-carbazol-4-yloxy)-2-propanol.

#### • ORGANIC IMPURITIES, PROCEDURE 3: CARVEDILOL RELATED COMPOUND F (if present)

**Solution A:** Trifluoroacetic acid and water (0.5:100)

**Solution B:** Methanol and trifluoroacetic acid (100:0.5)

**Diluent:** Water and acetonitrile (1:1)

**Mobile phase:** *Solution A* and *Solution B* (65:35)

**System suitability solution:** 1.5 mg/mL of USP

Carvedilol System Suitability Mixture RS in *Diluent*

**Sample solution:** 1.5 mg/mL of Carvedilol in *Diluent* prepared as follows. Initially add *Diluent* to fill about 80% of the total volume. Sonicate briefly to facilitate dissolution. Cool, and dilute with *Diluent* to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 226 nm

**Column:** 4.6-mm × 30-mm; 3-μm packing L7

**Column temperature:** 40°

**Flow rate:** 2 mL/min

**Injection size:** 10 μL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between carvedilol and carvedilol related compound F

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of carvedilol related compound F in the portion of the sample taken:

$$\text{Result} = (r_u/r_r) \times (1/F) \times 100$$

$r_u$  = peak response of carvedilol related compound F from the *Sample solution*

$r_r$  = sum of the peak responses of carvedilol and carvedilol related compound F from the *Sample solution*

$F$  = relative response factor, 1.1

**Acceptance criteria:** NMT 0.1%

#### SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 0.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* by HPLC other than *Procedure 1* is used, then the labeling states the test with which the article complies.



• **USP REFERENCE STANDARDS** (11)

USP Carvedilol RS

USP Carvedilol Related Compound A RS

1-(4-(2-Hydroxy-3-(2-(2-methoxyphenoxy)ethylamino)propoxy)-9H-carbazol-9-yl)-3-(2-(2-methoxyphenoxy)ethylamino)propan-2-ol.

 $C_{36}H_{43}N_3O_7$  629.74

USP Carvedilol Related Compound B RS

3,3'-(2-(2-Methoxyphenoxy)ethylazanediyl)bis(1-(9H-carbazol-4-yloxy)propan-2-ol).

 $C_{39}H_{39}N_3O_6$  645.74

USP Carvedilol Related Compound C RS

1-(9H-Carbazol-4-yloxy)-3-(benzyl(2-(2-methoxyphenoxy)ethylamino)propan-2-ol).

 $C_{31}H_{32}N_2O_4$  496.60

USP Carvedilol Related Compound D RS

4-(Oxiran-2-ylmethoxy)-9H-carbazole.

 $C_{15}H_{13}NO_2$  239.27

USP Carvedilol Related Compound E RS

2-(2-Methoxyphenoxy)ethyl amine.

 $C_9H_{13}NO_2$  167.21

USP Carvedilol System Suitability Mixture RS

Mixture of approximately 0.1% carvedilol related compound F (1-(2-(2-Methoxyphenoxy)ethylamino)-3-(2,3,4,9-tetrahydro-1H-carbazol-5-yloxy)propan-2-ol) in a matrix of carvedilol drug substance.

water, shake by hand, then add 70 mL of *Diluent*, and sonicate for 30 min. Shake on a mechanical shaker for about 30 min, and dilute with *Diluent* to volume to prepare a 0.25-mg/mL solution. Centrifuge an appropriate amount (about 50 mL) at 2000 rpm for 10 min.

**Sample solution:** 0.0125 mg/mL of carvedilol in *Methanol solution* from the *Sample stock solution*. Pass a portion of the solution through a suitable syringe filter of 0.45- $\mu$ m pore size, discard the first 5 mL, and use the filtrate as the *Sample solution*.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 240 nm**Column:** 4.6-mm  $\times$  50-mm; packing L7**Column temperature:** 40°**Flow rate:** 1 mL/min**Run time:** 30 min**Injection size:** 25  $\mu$ L**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of carvedilol ( $C_{24}H_{26}N_2O_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of the *Standard solution* (mg/mL) $C_U$  = nominal concentration of the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**• **DISSOLUTION** (711)**Test 1**

**Medium:** 0.7% (7 mL/L) of hydrochloric acid, adjusted with 50% (w/w) sodium hydroxide to a pH of 1.45  $\pm$  0.2; 900 mL; deaerated

**Apparatus 2:** 50 rpm**Time:** 30 min

**Standard stock solution:** Transfer about 7 mg of USP Carvedilol RS to a 250-mL volumetric flask. Add 5 mL of methanol, and sonicate until dissolved. Cool to room temperature, dilute with *Medium* to volume, and mix well.

**Standard solution:** On the basis of the label claim and using the *Standard stock solution*, prepare a solution of USP Carvedilol RS in *Medium* having an appropriate concentration ( $C_S$ ), as shown in Table 1.

Table 1

Label Claim (mg)	$C_S$ (mg/mL)
25	0.028
12.5	0.014
6.25	0.007
3.125	0.0035

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

**Analytical wavelengths:** 285 and 380 nm**Path length:** 1 cm**Blank:** *Medium*

**Analysis:** Calculate the corrected absorbance of the *Standard solution* and the *Sample solution*:

$$A_{\text{corr}} = A_{285} - A_{380}$$

**Carvedilol Tablets****DEFINITION**

Carvedilol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of carvedilol ( $C_{24}H_{26}N_2O_4$ ).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- B. ULTRAVIOLET ABSORPTION** (197U)  
Wavelength range: 250–400 nm  
Cell: 0.2 cm  
**Sample solution:** 0.125 mg/mL of carvedilol prepared as follows. Place 10 Tablets in a 150-mL polypropylene tube, and disintegrate the Tablets in methanol (100 mL for the Tablet strengths 3.125, 6.25, and 25 mg, and 50 mL for the Tablet strength 12.5 mg) using a mechanical homogenizer. Transfer the homogenate to an appropriate volumetric flask, and dilute with methanol to volume. Pass through a suitable PTFE filter of 0.45- $\mu$ m pore size.

**ASSAY**• **PROCEDURE**

**Buffer:** Dissolve 0.7 g of anhydrous monobasic potassium phosphate in 500 mL of water, and add 10 mL of triethylamine. Adjust with phosphoric acid to a pH of 3.0  $\pm$  0.1.

**Mobile phase:** Dissolve 1.04 g of sodium dodecyl sulfate in 150 mL of *Buffer* in a 2-L volumetric flask, and sonicate. Add 720 mL of acetonitrile, and dilute with water to volume. Pass through a nylon 66 filter of 0.2- $\mu$ m pore size.

**Diluent:** Methanol and 1 M hydrochloric acid (9:1)**Methanol solution:** Methanol and water (1:1)

**Standard solution:** 0.0125 mg/mL of USP Carvedilol RS prepared as follows. Dissolve a quantity of USP Carvedilol RS in a mixture of *Diluent* and water (9:1), and sonicate until the solution is clear. Dilute with *Methanol solution* to obtain the required final concentration.

**Sample stock solution:** Transfer a portion of the powdered Tablets (NLT 20), equivalent to 25 mg of carvedilol, to a 100-mL volumetric flask. Add 10 mL of



$A_{corr}$  = corrected absorbance of the *Standard solution* or the *Sample solution*  
 $A_{285}$  = absorbance of the *Standard solution* or the *Sample solution* at 285 nm  
 $A_{380}$  = absorbance of the *Standard solution* at 380 nm  
 Calculate the percentage of carvedilol dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (V/L) \times 100$$

$A_U$  = corrected absorbance from the *Sample solution*  
 $A_S$  = corrected absorbance from the *Standard solution*  
 $C_S$  = corrected concentration of the *Standard solution* (mg/mL)  
 $V$  = volume of *Medium*, 900 mL  
 $L$  = label claim (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of carvedilol ( $C_{24}H_{26}N_2O_4$ ) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** Simulated gastric fluid without enzymes; 900 mL

**Apparatus 2, Time, Standard stock solution, Standard solution, Sample solution, and Analysis:** Proceed as directed in *Test 1*.

**Tolerances:** NLT 80% (Q) of the labeled amount of carvedilol ( $C_{24}H_{26}N_2O_4$ ) is dissolved.

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium:** Simulated gastric fluid with pepsin, pH 1.45 (dissolve 12.0 g of sodium chloride and 19.2 g of purified pepsin (porcine origin, activity 800–2500 Units/mg of protein) in 18 mL of hydrochloric acid and sufficient water to make 6 L; adjust with hydrochloric acid to a pH of 1.45); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Buffer:** 2.72 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of  $2.0 \pm 0.05$ .

**Mobile phase:** Buffer and acetonitrile (650:350)

**Standard stock solution:** 1.4 mg/mL of USP Carvedilol RS in methanol

**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration of ( $L/900$ ) mg/mL, where  $L$  is the Tablet label claim, in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm  $\times$  15-mm; 5- $\mu$ m packing L7

**Column temperature:** 35°

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 3500 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis:** Calculate the percentage of carvedilol dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)  
 $L$  = label claim (mg/Tablet)  
 $V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of carvedilol ( $C_{24}H_{26}N_2O_4$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905)

**Buffer, Mobile phase, Diluent, Methanol solution, Standard solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.  
**Sample solution:** 0.25 mg/mL of carvedilol prepared as follows. Place 1 Tablet into a volumetric flask of appropriate size, based on the label claim. Add water to the flask up to about 10% of volume, and shake by hand to disintegrate the Tablet. Fill the flask up to 75% of volume with *Diluent*, and sonicate for 30 min to obtain complete disintegration. Shake on a mechanical shaker for 30 min, allow to cool, and dilute with *Diluent* to volume. Centrifuge an appropriate amount of this solution for 10 min at 2400 rpm, and transfer 4 mL of supernatant into a 100-mL volumetric flask. Fill the flask to about 85% of volume with *Methanol solution*, and sonicate for 20 min, with intermittent shaking. Dilute with *Methanol solution* to volume, and pass through a suitable syringe filter of 0.45- $\mu$ m pore size.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of carvedilol ( $C_{24}H_{26}N_2O_4$ ) in the Tablet taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Buffer, Mobile phase, Diluent, Methanol solution, and Sample stock solution:** Prepare as directed in the *Assay*.

**Standard stock solution:** Use the *Standard solution* from the *Assay*.

**Standard solution:** 1.25  $\mu$ g/mL USP Carvedilol RS in a mixture of *Diluent* and water (1:1) from the *Standard stock solution*

**Sample solution:** Dilute with water to volume, 25 mL of the supernatant from the *Sample stock solution* in a 50-mL volumetric flask. Pass a portion of the solution through a suitable syringe filter of 0.45- $\mu$ m pore size.

**Chromatographic system:** Proceed as directed in the *Assay*, except for *Injection size*.

**Injection size:** 15  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of carvedilol from the *Standard solution*  
 $C_S$  = concentration of USP Carvedilol RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of carvedilol in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** NMT 0.2% (specified or unspecified)



**Total impurities:** NMT 1.0%

[NOTE—Disregard any peaks with a relative retention time less than or equal to 0.04 and peaks with less than 0.05% of the nominal carvedilol peak response in the *Sample solution*.]

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers protected from moisture. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**  
USP Carvedilol RS

### Casanthranol

» Casanthranol is obtained from *Cascara Sagrada*. It contains in each 100 g not less than 20.0 g of total hydroxyanthracene derivatives calculated on the dried basis, calculated as cascaroside A. Not less than 80 percent of the total hydroxyanthracene derivatives consists of cascarosides, calculated as cascaroside A.

**Packaging and storage—**Preserve in tight, light-resistant containers, at a temperature not exceeding 30°.

**Loss on drying (731)—**Dry it in vacuum at 80° for 16 hours: it loses not more than 10.0% of its weight.

**Residue on ignition (281):** not more than 4.0%.

**Delete the following:**

• **Heavy metals, Method II (231):** 0.0025%. (Official 1-Jan-2018)

**Assay for total hydroxyanthracene derivatives—**[NOTE 1—Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results. NOTE 2—Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed for *Volumetric Solutions* in the section *Reagents, Indicators, and Solutions*.]

**Ferric chloride solution—**Dissolve 100 g of ferric chloride in water to make 100 mL.

**Assay solution—**Mix a portion of Casanthranol, and transfer an accurately weighed quantity of about 500 mg to a 100-mL volumetric flask. Add about 30 mL of 70 percent alcohol, swirl to dissolve, dilute with 70 percent alcohol to volume, and mix. Quickly filter through soft, rapid-flow filter paper, taking precautions to minimize loss by evaporation.

**Assay preparation—**Pipet 10 mL of *Assay solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water, to a 50-mL volumetric flask, filtering through a small pledget of cotton, water-wet, dilute with water to volume, and mix.

**Procedure—**Pipet 10 mL of *Assay preparation* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 hours by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 minutes, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 1 in 200 solution of magnesium acetate in methanol. Determine the absorbance against methanol as a reference, in 1-cm cells at the wavelength of maximum absorbance at about 515 nm. Calculate the quantity, in mg, of total hydroxyanthracene derivatives in the portion of Casanthranol taken by the formula:

$$155A_U$$

in which  $A_U$  is the absorbance of the solution from the *Assay preparation*.

**Assay for cascarosides—**[NOTE 1—Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results. NOTE 2—Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed for *Volumetric Solutions* in the section *Reagents, Indicators, and Solutions*.]

**Ferric chloride solution and Assay solution—**Prepare as directed in the *Assay for total hydroxyanthracene derivatives*.

**Assay preparation—**Pipet 10 mL of *Assay solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask, filtering through a small pledget of cotton, water-wet, dilute with water to volume, and mix.

**Procedure—**Pipet 15 mL of *Assay preparation* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 hours by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory



funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 minutes, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 1 in 200 solution of magnesium acetate in methanol. Determine the absorbance, against methanol as a reference, in 1-cm cells at the wavelength of maximum absorbance at about 515 nm. Calculate the quantity, in mg, of cascarosides in the portion of Casanthranol taken by the formula:

$$103.5A_U$$

in which  $A_U$  is the absorbance of the solution from the Assay preparation.

## Cascara Sagrada

### DEFINITION

Cascara Sagrada is the dried bark of *Frangula purshiana* (DC.) J. G. Cooper (syn. *Rhamnus purshiana* DC.) (Fam. Rhamnaceae). It yields NLT 7.0% of total hydroxyanthracene derivatives, calculated as cascaroside A, and calculated on the dried basis. NLT 60% of the total hydroxyanthracene derivatives consists of cascarosides, calculated as cascaroside A.

[NOTE—Collect Cascara Sagrada not less than one year before use.]

### IDENTIFICATION

- **A.**  
**Sample:** 100 mg of powdered Cascara Sagrada  
**Analysis:** Add the *Sample* to 10 mL of hot water, shake the mixture occasionally until it is cold, filter, dilute the filtrate with water to 10 mL, and add 10 mL of 6 N ammonium hydroxide.  
**Acceptance criteria:** An orange color is produced.
- **B.**  
**Sample:** A portion of Cascara Sagrada  
**Analysis:** Treat the *Sample* with 6 N ammonium hydroxide.  
**Acceptance criteria:** It becomes red to reddish brown in color.
- **C.**  
**Sample:** 100 mg of powdered Cascara Sagrada  
**Analysis:** Macerate the *Sample* with 1 mL of alcohol, add 10 mL of water, boil the mixture, then cool, filter, and shake the filtrate with 10 mL of ether: a greenish-yellow ether layer separates. Shake 3 mL of the ether layer with 3 mL of 6 N ammonium hydroxide, and dilute the separated ammonia solution with 20 mL of water.  
**Acceptance criteria:** A distinct orange-pink color remains.

### COMPOSITION

#### • CONTENT OF TOTAL HYDROXYANTHRACENE DERIVATIVES

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this procedure, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions*.

**Ferric chloride solution:** 1 g/mL of ferric chloride in water

**Sample stock solution:** Add 1 g of Cascara Sagrada to 70 mL of boiling water, boil for several min, with stirring. Allow to cool, and transfer with the aid of water to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter through suitable filter paper.

**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water, to a 50-mL volumetric flask, dilute with water to volume, and mix.

#### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** Visible

**Analytical wavelength:** 515 nm

**Cell:** 1 cm

**Blank:** Methanol

#### Analysis

**Sample:** *Sample solution*

Pipet 15 mL of *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 15.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5-mg/mL solution of magnesium acetate in methanol.

Calculate the quantity, in mg, of total hydroxyanthracene derivatives ( $T_{HD}$ ) in the portion of Cascara Sagrada taken:

$$T_{HD} = A_U \times F$$

$A_U$  = absorbance of the *Sample solution*

$F$  = conversion factor, 138. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A and the dilutions to prepare the solution for analysis.]

Calculate the percentage of total hydroxyanthracene derivatives, calculated as cascaroside A:

$$\text{Result} = (T_{HD}/W) \times 100$$

$W$  = weight of Cascara Sagrada taken to prepare the *Sample stock solution* (mg)

**Acceptance criteria:** NLT 7.0%, calculated on the dried basis

#### • CONTENT OF CASCAROSIDES

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.



Throughout this procedure, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions*.

**Ferric chloride solution and Sample stock solution:**

Prepare as directed in the *Assay for Content of Total Hydroxyanthracene Derivatives*.

**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared, water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared, water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared, water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask. Dilute with water to volume.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Visible

Analytical wavelength: 515 nm

Cell: 1 cm

Blank: Methanol

**Analysis**

Proceed as directed for *Analysis* in *Content of Total Hydroxyanthracene Derivatives*, except to evaporate a 20.0-mL portion of the methylene chloride solution instead of 15.0 mL.

**Sample:** *Sample solution*

Determine the absorbance and calculate the percentage of cascarosides with respect to the content of total hydroxyanthracene derivatives in the portion of Cascara Sagrada taken:

$$\text{Result} = (A_U / T_{HD}) \times F \times 100$$

$A_U$  = absorbance of the *Sample solution*

$T_{HD}$  = weight of total hydroxyanthracene derivatives (mg)

$F$  = conversion factor, 103.5. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A and the dilutions to prepare the solution for analysis.]

**Acceptance criteria:** NLT 60% of the total hydroxyanthracene derivatives consists of cascarosides, calculated as cascaroside A on the dried basis

**SPECIFIC TESTS**

• **BOTANIC CHARACTERISTICS**

**Macroscopic**

**Cascara Sagrada:** The bark is usually in the form of flattened or transversely curved pieces, occasionally in quills of variable length and from 1 to 5 mm in thickness. The outer surface is brown, purplish brown, or brownish red, longitudinally ridged, with or without grayish or whitish lichen patches, sometimes with numerous lenticels and occasionally with moss attached. The inner surface is longitudinally striate, light yellow, weak reddish brown, or moderate yellowish brown.

The fracture is short with projections of phloem fiber bundles in the inner bark.

**Powdered Cascara Sagrada:** The powder is moderate yellowish brown to dusky yellowish orange.

**Microscopic**

**Cascara Sagrada:** The transverse section of the bark shows a yellowish-brown, purple, or reddish-brown cork of up to 10 or more rows of small cells; stone cells in yellowish, tangentially elongated groups of 20–50 cells in the cortex, pericycle, and outer phloem regions; phloem rays 1–4 cells wide, 15–25 cells deep, frequently diagonal or curved, forming converging groups; phloem fibers in small bundles, more or less surrounded by crystal fibers and located between the phloem rays; parenchyma with brown walls and containing starch grains and calcium oxalate crystals.

**Powdered Cascara Sagrada:** It shows numerous broken phloem fiber bundles with accompanying crystal fibers containing monoclinic prisms of calcium oxalate; stone cells more or less adherent, in small groups with thick, finely lamellated and porous walls; fragments of reddish-brown to yellow cork; masses of parenchyma and phloem ray cells colored reddish brown to orange upon the addition of a solution of an alkali; starch grains spheroidal, up to 8 µm in diameter; calcium oxalate in monoclinic prisms or rosette aggregates from 6 to 20 µm in diameter, occasionally up to 45 µm in diameter.

• **ARTICLES OF BOTANICAL ORIGIN, Foreign Organic Matter** (561): NMT 4.0%

• **WATER DETERMINATION, Method III, Procedure for Articles of Botanical Origin** (921): Dry a sample at 105° for 5 h: it loses NMT 12.0% of its weight.

## Cascara Sagrada Extract

**DEFINITION**

Cascara Sagrada Extract contains, in each 100 g, NLT 10.0 g and NMT 12.0 g of total hydroxyanthracene derivatives, of which NLT 50% consists of cascarosides, both calculated as cascaroside A.

Mix 900 g of Cascara Sagrada, in coarse powder, with 4000 mL of boiling water, and macerate the mixture for 3 h. Then transfer it to a percolator, allow it to drain, exhaust it by percolation, using boiling water as the menstruum, and collect 5000 mL of percolate. Evaporate the percolate to dryness, reduce the Extract to a fine powder, and, after assaying, add sufficient starch, dried at 100°, or other inert, nontoxic diluents to make the product contain, in each 100 g, 11 g of hydroxyanthracene derivatives. Mix the powders, and pass the Extract through a number 60 sieve.

**ASSAY**

• **CONTENT OF TOTAL HYDROXYANTHRACENE DERIVATIVES**

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions*.

**Ferric chloride solution:** 1 g/mL of ferric chloride in water

**Sample stock solution:** Transfer 1 g of Extract to a 100-mL volumetric flask. Add 60 mL of 70% alcohol, swirl or sonicate for 15–20 min several times, and allow to stand overnight. Sonicate or swirl for 10–15 min, dilute with 70% alcohol to volume, mix, and filter through suitable filter paper.



**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water, to a 50-mL volumetric flask, and dilute with water to volume, and mix.

#### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Visible

Analytical wavelength: 515 nm

Cell: 1 cm

Blank: Methanol

#### Analysis

**Sample:** *Sample solution*

**Analysis:** Pipet 10 mL of *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, and dilute with methylene chloride to volume. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5 mg/mL solution of magnesium acetate in methanol.

Calculate the quantity, in mg, of total hydroxyanthracene derivatives ( $T_{HD}$ ) in the portion of Cascara Sagrada Extract taken:

$$T_{HD} = A_U \times F$$

$A_U$  = absorbance of the *Sample solution*

$F$  = conversion factor, 155.2. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A and the dilutions to prepare the solution for analysis.]

Calculate the percentage of total hydroxyanthracene derivatives in the portion of Cascara Sagrada Extract taken:

$$\text{Result} = (T_{HD}/W) \times 100$$

$W$  = weight of Cascara Sagrada Extract taken to prepare the *Sample stock solution* (mg)

**Acceptance criteria:** 10.0%–12.0% of total hydroxyanthracene derivatives, calculated as cascaroside A

#### • CONTENT OF CASCAROSIDES

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions—Volumetric Solutions*.

#### Ferric chloride solution and Sample stock solution:

Prepare as directed in the *Assay for Content of Total Hydroxyanthracene Derivatives*.

**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared, water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared, water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared, water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask. Dilute with water to volume, and mix.

#### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Visible

Analytical wavelength: 515 nm

Cell: 1 cm

Blank: Methanol

#### Analysis

**Sample:** *Sample solution*

Pipet 25 mL of the *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix. Evaporate a 20.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5-mg/mL solution of magnesium acetate in methanol.

Determine the absorbance and calculate the percentage of cascarosides with respect to the content of total hydroxyanthracene derivatives in the portion of Cascara Sagrada Extract taken:

$$\text{Result} = (A_U/T_{HD}) \times F \times 100$$

$A_U$  = absorbance of the *Sample solution*

$T_{HD}$  = weight of total hydroxyanthracene derivatives (mg)

$F$  = conversion factor, 62.06. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A and the dilutions to prepare the solution for analysis.]

**Acceptance criteria:** NLT 50% of total hydroxyanthracene derivatives calculated as cascaroside A



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature not exceeding 30°.

**Cascara Tablets****DEFINITION**

Cascara Tablets are prepared from Cascara Sagrada Extract. They contain NLT 9.35% and NMT 12.65% of total hydroxyanthracene derivatives, calculated as cascarioside A, in the labeled amount of Cascara Sagrada Extract. NLT 50% of the hydroxyanthracene derivatives are cascariosides, calculated as cascarioside A.

**STRENGTH**• **CONTENT OF TOTAL HYDROXYANTHRACENE DERIVATIVES**

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.6 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions, Volumetric Solutions*.

**Ferric chloride solution:** 1 g/mL of ferric chloride in water

**Sample stock solution:** Weigh and finely powder NLT 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to 1 g of Cascara Sagrada Extract, to a 100-mL volumetric flask. Add 60 mL of 70% alcohol, swirl or sonicate for 15–20 min several times, and allow to stand overnight. Sonicate or swirl for 10–15 min, dilute with 70% alcohol to volume, mix, and filter through suitable filter paper.

**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the combined water layers, with the aid of water, to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** Visible

**Analytical wavelength:** 515 nm

**Cell:** 1 cm

**Blank:** Methanol

**Analysis**

**Sample:** *Sample solution*

Pipet 10 mL of *Sample solution* into a flask containing 2 mL of *Ferric chloride solution* and 12 mL of hydrochloric acid. Attach a condenser arranged for refluxing, and heat for 3 h by keeping the flask immersed in boiling water or continuously exposed to steam heat. Cool, wash down the condenser, and transfer to a separatory funnel with the aid of 4 mL of 1 N sodium hydroxide and five 6-mL portions of water. Extract with 20 mL of methylene chloride, and transfer the lower layer to another separatory funnel. Repeat the extraction with three additional 20-mL portions of methylene chloride, wash the combined methylene chloride

extracts with two 10-mL portions of water, shaking each time for 2 min, and discard the water washings. Transfer the washed methylene chloride extract to a 100-mL volumetric flask, dilute with methylene chloride to volume, and mix.

Evaporate a 15.0-mL portion carefully on a water bath to dryness, and dissolve the residue in 10.0 mL of a 5-mg/mL solution of magnesium acetate in methanol. Calculate the quantity, in mg, of total hydroxyanthracene derivatives ( $T_{HD}$ ) in the portion of Cascara Sagrada Extract taken:

$$T_{HD} = A_U \times F$$

$A_U$  = absorbance of the *Sample solution*

$F$  = conversion factor, 206.9. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascarioside A, and the dilutions to prepare the solution for analysis.]

Calculate the percentage of total hydroxyanthracene derivatives in the nominal amount of Cascara Sagrada Extract taken:

$$\text{Result} = (T_{HD}/W) \times 100$$

$W$  = nominal weight of Cascara Sagrada Extract in the portion of Tablets powder taken to prepare the *Sample stock solution* (mg)

**Acceptance criteria:** 9.35%–12.65% in the labeled amount of Cascara Sagrada Extract, calculated as cascarioside A

• **CONTENT OF CASCARIOSIDES**

Perform all extractions by shaking vigorously, and allow all phases to separate completely before transferring. Entrainment of aglycones into the aqueous phase, as indicated by a value of less than 2.7 for the ratio of the absorbance of the final solution at 515 nm to that at 440 nm, may lead to false results.

Throughout this assay, use 1 N sodium hydroxide that is prepared without added barium ions as directed in *Reagents, Indicators, and Solutions, Volumetric Solutions*.

**Ferric chloride solution and Sample stock solution:**

Prepare as directed in *Content of Total Hydroxyanthracene Derivatives*.

**Sample solution:** Pipet 10 mL of *Sample stock solution* into a separatory funnel containing 5 mL of water and 2 drops of 1 N hydrochloric acid. Extract with 40 mL of methylene chloride, and transfer the lower layer to a second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, discard the lower layer, and transfer the water layer to the first separatory funnel. Extract the combined water layers with 40 mL of methylene chloride, and transfer the lower layer to the second separatory funnel. Add 10 mL of water to the second separatory funnel, and shake. Allow to separate, and discard the lower layer. Transfer the water layer to the first separatory funnel. Extract the combined aqueous phase with 30 mL of clear, freshly prepared, water-saturated ethyl acetate, and transfer the water layer to another separatory funnel. Repeat the extraction with two additional 30-mL portions of the freshly prepared, water-saturated ethyl acetate. Add 5 mL of water to the combined ethyl acetate extracts, shake, allow the phases to separate, discard the ethyl acetate extracts, and add 30 mL of the freshly prepared, water-saturated ethyl acetate to the water wash. Shake, allow the phases to separate, and discard the ethyl acetate phase. Transfer the combined aqueous phases, with the aid of water, to a 50-mL volumetric flask. Dilute with water to volume, and mix.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)



Mode: Visible  
 Analytical wavelength: 515 nm  
 Cell: 1 cm  
 Blank: Methanol

**Analysis****Sample:** *Sample solution*

Prepare as directed for *Analysis* in *Content of Total Hydroxyanthracene Derivatives*, except to pipet 20 mL of *Sample solution*.

Determine the absorbance, and calculate the percentage of cascarosides with respect to the content of total hydroxyanthracene derivatives in the nominal amount of Cascara Sagrada Extract in the portion of Tablets powder taken:

$$\text{Result} = (A_U/T_{HD}) \times F \times 100$$

$A_U$  = absorbance of the *Sample solution*  
 $T_{HD}$  = weight of total hydroxyanthracene derivatives (mg)  
 $F$  = conversion factor, 103.5. [NOTE—This conversion factor considers an absorptivity of 16.1 for cascaroside A, and the dilutions to prepare the solution for analysis.]

**Acceptance criteria:** NLT 50% of the content of total hydroxyanthracene derivatives are cascarosides, calculated as cascaroside A.

**PERFORMANCE TESTS**

- **DISINTEGRATION** (701): 60 min
- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers; if the Tablets are coated, well-closed containers may be used.

**Cascara Sagrada Fluidextract****DEFINITION**

Prepare Cascara Sagrada Fluidextract as follows. To 1000 g of coarsely ground Cascara Sagrada add 3000 mL of boiling water, and allow to macerate in a suitable percolator for 2 h. Allow the percolation to proceed at a moderate rate, gradually adding boiling water until the drug is practically exhausted of its active principles. Evaporate the percolate on a water bath or in a vacuum still to NMT 800 mL. Cool, add 200 mL of alcohol and, if necessary, add sufficient water to make the product measure 1000 mL. Mix.

**OTHER COMPONENTS**

- **ALCOHOL DETERMINATION, Method I** (611): 18.0%–20.0% of  $C_2H_5OH$

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

**Aromatic Cascara Fluidextract****DEFINITION**

Prepare Aromatic Cascara Fluidextract as follows.

Cascara Sagrada, as a very coarse powder	1000 g
Magnesium Oxide	120 g

Suitable sweetening agent(s)	
Suitable essential oils(s)	
Suitable flavoring agent(s)	
Alcohol	200 mL
Purified Water, a sufficient quantity, to make	1000 mL

Mix the Cascara Sagrada with *Magnesium Oxide*, moisten it uniformly with 2000 mL of boiling water, and set it aside in a shallow container for 48 h, stirring occasionally. Pack it in a percolator, and percolate with boiling water until the material is exhausted. Evaporate the percolate, at a temperature not exceeding 100°, to 750 mL, and at once dissolve in it the flavoring agent(s). When the liquid has cooled, add the *Alcohol*, in which the sweetening agent(s) and oils have been dissolved, and add sufficient water to make the Aromatic Fluidextract measure 1000 mL. Mix.

**OTHER COMPONENTS**

- **ALCOHOL DETERMINATION** (611): 18%–20% of  $C_2H_5OH$ , determined by the gas-liquid chromatographic method, using acetone as the internal standard

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and avoid exposure to direct sunlight and to excessive heat.

**Castor Oil****DEFINITION**

Castor Oil is the fixed oil obtained from the seed of *Ricinus communis* L. (Fam. Euphorbiaceae). It contains no added substances.

**IMPURITIES****Delete the following:**

- **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1-Jan-2018)

**SPECIFIC TESTS**

- **SPECIFIC GRAVITY** (841): 0.957–0.961
- **DISTINCTION FROM MOST OTHER FIXED OILS:** It is only partly soluble in solvent hexane (distinction from most other fixed oils), but it yields a clear liquid with an equal volume of alcohol (foreign fixed oils).
- **FATS AND FIXED OILS, Free Fatty Acids** (401): The free fatty acids in 10 g require NMT 3.5 mL of 0.10 N sodium hydroxide for neutralization.
- **FATS AND FIXED OILS, Hydroxyl Value** (401)  
 Free acid determination  
 Sample: 10 g  
 Titrimetric system  
 (See *Titrimetry* (541).)  
 Mode: Direct titration  
 Titrant: 0.5 N alcoholic potassium hydroxide VS  
 Endpoint detection: Visual  
 Analysis: Transfer the *Sample* to a 250-mL conical flask, add 10 mL of pyridine that has been neutralized previously to phenolphthalein, and swirl to mix. Add 1 mL of phenolphthalein TS, and titrate with *Titrant* to a faint pink endpoint. Record the volume of *Titrant* consumed ( $V_A$ ).  
 Hydroxyl value determination  
 Sample: 2 g  
 Blank: 5.0 mL of a freshly prepared mixture of 1 volume of acetic anhydride and 3 volumes of pyridine



**Titrimetric system**(See *Titrimetry* (541).)**Mode:** Residual titration**Titrant:** 0.5 N alcoholic potassium hydroxide VS**Endpoint detection:** Visual

**Analysis:** Transfer the *Sample* to a glass-stoppered, 250-mL conical flask. Add 5.0 mL of a freshly prepared mixture of 1 volume of acetic anhydride and 3 volumes of pyridine, and swirl to mix. Connect the flask to a reflux condenser, and heat on a steam bath for 2 h. Add 10 mL of water through the condenser, swirl to mix, heat on a steam bath for an additional 10 min, and allow to cool to room temperature. Add through the condenser 15 mL of normal butyl alcohol that has been neutralized previously to phenolphthalein, remove the condenser, and wash the tip of the condenser and the sides of the flask with an additional 10 mL of neutralized normal butyl alcohol. Add 1 mL of phenolphthalein TS, and titrate with *Titrant* to a faint pink endpoint.

Calculate the hydroxyl value in the portion of Oil taken:

$$\text{Result} = [(V_B + (W \times V_A/W_A) - V_T) \times M_r \times N]/W$$

$V_B$  = volume of *Titrant* consumed by the *Blank* (mL)

$W$  = sample weight from the hydroxyl value determination (g)

$V_A$  = volume of *Titrant* consumed by the *Sample* in the free acid determination (mL)

$W_A$  = sample weight from the free acid determination (g)

$V_T$  = volume of *Titrant* consumed by the *Sample* in the hydroxyl value determination (mL)

$M_r$  = milliequivalent weight of potassium hydroxide, 56.11 mg/mEq

$N$  = actual normality of the *Titrant*

**Acceptance criteria:** 160–168

- **FATS AND FIXED OILS, Iodine Value (401):** 83–88
- **FATS AND FIXED OILS, Saponification Value (401):** 176–182

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.

**Castor Oil Capsules****DEFINITION**

Castor Oil Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of castor oil, calculated from the tests for *Weight Variation* and *Specific Gravity*.

**IDENTIFICATION**• **A. INFRARED ABSORPTION (197S)**

**Standard solution:** 40 mg/mL of Castor Oil in chloroform

**Sample solution:** 40 mg/mL of the oil from Capsules in chloroform

**Acceptance criteria:** Meet the requirements

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**SPECIFIC TESTS**• **SPECIFIC GRAVITY (841)**

**Sample:** Capsule contents

**Acceptance criteria:** 0.957–0.961

- **FATS AND FIXED OILS, Free Fatty Acids (401):** The free fatty acids in 10 g require NMT 3.5 mL of 0.10 N sodium hydroxide for neutralization.

• **FATS AND FIXED OILS, Hydroxyl Value (401)**

**Free acid determination:** Determine the amount of free acid in the Capsule contents.

**Sample:** 10 g of Capsule contents

**Titrimetric system**(See *Titrimetry* (541).)**Mode:** Direct titration**Titrant:** 0.5 N alcoholic potassium hydroxide VS**Endpoint detection:** Visual

**Analysis 1:** Transfer the *Sample* to a 250-mL conical flask, add 10 mL of pyridine that has been neutralized previously to phenolphthalein, and swirl to mix. Add 1 mL of phenolphthalein TS, and titrate with *Titrant* to a faint pink endpoint. Record the volume of *Titrant* consumed.

**Hydroxyl value determination:** Determine the hydroxyl value of the Capsule contents.

**Sample:** 2 g from the Capsule contents

**Blank:** 5.0 mL of a freshly prepared mixture of 1 volume of acetic anhydride and 3 volumes of pyridine

**Titrimetric system**(See *Titrimetry* (541).)**Mode:** Residual titration**Titrant:** 0.5 N alcoholic potassium hydroxide VS**Endpoint detection:** Visual

**Analysis 2:** Transfer the *Sample* to a glass-stoppered, 250-mL conical flask. Add 5.0 mL of a freshly prepared mixture of 1 volume of acetic anhydride and 3 volumes of pyridine, and swirl to mix. Connect the flask to a reflux condenser, and heat on a steam bath for 2 h. Add 10 mL of water through the condenser, swirl to mix, heat on a steam bath for an additional 10 min, and allow to cool to room temperature. Add through the condenser 15 mL of normal butyl alcohol that previously has been neutralized to phenolphthalein, remove the condenser, and wash the tip of the condenser and the sides of the flask with an additional 10 mL of neutralized normal butyl alcohol. Add 1 mL of phenolphthalein TS, and titrate with *Titrant* to a faint pink endpoint.

Calculate the hydroxyl value in the portion of Capsule contents taken:

$$\text{Result} = (M_r \times N/W) \times [B + (W \times A/C) - T]$$

$M_r$  = milliequivalent weight of potassium hydroxide, 56.11 mg/mEq

$N$  = actual normality of the *Titrant*

$W$  = *Sample* weight for the hydroxyl determination (g)

$B$  = *Titrant* volume consumed by the *Blank* (mL)

$A$  = *Titrant* volume consumed by the *Sample* in the free acid determination (mL)

$C$  = *Sample* weight for the free acid determination (g)

$T$  = *Titrant* volume consumed by the *Sample* in the hydroxyl determination (mL)

**Acceptance criteria:** 160–168 in mg of KOH/g of Capsule content or hydroxyl value

• **FATS AND FIXED OILS, Iodine Value (401)**

**Sample:** Capsule contents

**Acceptance criteria:** 83–88

• **FATS AND FIXED OILS, Saponification Value (401)**

**Sample:** Capsule contents

**Acceptance criteria:** 176–182

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably at controlled room temperature.



## Castor Oil Emulsion

### DEFINITION

Castor Oil Emulsion contains NLT 90.0% and NMT 120.0% of the labeled amount of Castor Oil.

### IDENTIFICATION

#### • A.

**Sample:** 10 mL of Emulsion well shaken

**Analysis:** Transfer the *Sample* to a 125-mL separator. Add 10 mL of 1 N hydrochloric acid and 20 mL of solvent hexane. Shake vigorously for 2–3 min, allow the layers to separate, discard the aqueous phase, and filter the upper layer through anhydrous sodium sulfate into a small beaker. Evaporate the solvent on a steam bath, and to the residue add 1–2 drops of sulfuric acid.

**Acceptance criteria:** A red color indicates the presence of castor oil.

### ASSAY

#### • PROCEDURE

**Internal standard solution:** 12 mg/mL of di(2-ethylhexyl)phthalate in chloroform

**Standard solution:** Transfer 100 mg of castor oil to a 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid, reflux in a water bath maintained at 75°–80° for 2.5 h, cool, and rinse down the condenser with 10 mL of water. Transfer the contents of the flask to a 125-mL separator with the aid of 10 mL of water. Rinse the condenser and the flask with 25 mL of solvent hexane, and transfer to the separator. Shake the separator for 2 min, and draw off the aqueous layer into a second 125-mL separator. Add 20 mL of solvent hexane to the second separator, shake for 2 min, discard the aqueous layer, and transfer the solvent hexane layer to the first separator with the aid of 10 mL of solvent hexane. Wash the combined extracts with three 5-mL portions of water, discarding the washings, and transfer the washed extract to a 125-mL conical flask through a funnel containing anhydrous sodium sulfate, with the aid of 25 mL of solvent hexane. Place the flask in a hot water bath, and evaporate with the aid of a current of air to dryness. To the residue add 10.0 mL of *Internal standard solution*, and mix until solution is complete.

**Sample solution:** Transfer an amount of Emulsion, well-shaken and nominally equivalent to 100 mg of castor oil, to a long-neck, round-bottom 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Prepare as directed in *Standard solution*, beginning with "Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid".

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 1.8-m × 4-mm; packed with 4% liquid phase G25 on support S1

**Column conditioning:** Flush with helium for 2–5 min, then heat without further flushing at 250° for NLT 30 min, then cool to room temperature, and finally heat while helium is flowing through it at 250° for NLT 60 min.

#### Temperature

**Column:** 245°

**Injector:** 300°

**Detector:** 300°

**Flow rate:** Adjust to obtain a peak due to castor oil 5.5 min after introduction of the specimen and an in-

ternal standard peak 8 min after introduction of the specimen.

**Carrier gas:** Helium

**Injection size:** 5 µL

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Measure the heights of the peaks due to castor oil and the internal standard.

Calculate the percentage of castor oil in the portion of Emulsion taken:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times 100$$

$R_U$  = ratio of the heights of the peaks due to castor oil and internal standard, *Sample solution*

$R_S$  = ratio of the heights of the peaks due to castor oil and internal standard, *Standard solution*

$W_S$  = weight of castor oil taken to prepare the *Standard solution* (mg)

$W_U$  = nominal weight of castor oil in the amount of Emulsion taken to prepare the *Sample solution* (mg)

**Acceptance criteria:** 90.0%–120.0%

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

## Aromatic Castor Oil

### DEFINITION

Aromatic Castor Oil is Castor Oil containing suitable flavoring agents. It contains NLT 95.0% of castor oil.

### ASSAY

#### • PROCEDURE

**Internal standard solution:** 12 mg/mL of di(2-ethylhexyl)phthalate in chloroform

**Standard solution:** Transfer 100 mg of Castor Oil to a 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid, reflux in a water bath maintained at 75°–80° for 2.5 h, cool, and rinse down the condenser with 10 mL of water. Transfer the contents of the flask to a 125-mL separator with the aid of 10 mL of water. Rinse the condenser and the flask with 25 mL of solvent hexane, and transfer to the separator. Shake the separator for 2 min, and draw off the aqueous layer into a second 125-mL separator. Add 20 mL of solvent hexane to the second separator, shake for 2 min, discard the aqueous layer, and transfer the solvent hexane layer to the first separator with the aid of 10 mL of solvent hexane. Wash the combined extracts with three 5-mL portions of water, discarding the washings, and transfer the washed extract to a 125-mL conical flask, through a funnel containing anhydrous sodium sulfate, with the aid of 25 mL of solvent hexane. Place the flask in a hot water bath, and evaporate with the aid of a current of air to dryness. To the residue add 10.0 mL of *Internal standard solution*, and mix until solution is complete.

**Sample solution:** Transfer an amount of Aromatic Castor Oil, well-shaken and nominally equivalent to 100 mg of castor oil, to a long-neck, round-bottom 100-mL boiling flask equipped with a suitable reflux condenser connected by a ground-glass joint. Proceed as directed for the *Standard solution*, beginning with "Add 30 mL of a mixture of 300 mL of methanol and 3.7 mL of sulfuric acid...".



**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** GC**Detector:** Flame ionization**Column:** 1.8-m × 4-mm column packed with 4% liquid phase G25 on support S1**Column conditioning:** Flush with helium for 2–5 min, then heat without further flushing at 250° for NLT 30 min, then cool to room temperature, and finally heat while helium is flowing through it at 250° for NLT 60 min.**Temperature****Column:** 245°**Injector:** 300°**Detector:** 300°**Flow rate:** Adjust to obtain a peak due to castor oil 5.5 min after introduction of the specimen and an internal standard peak 8 min after introduction of the specimen.**Carrier gas:** Helium**Injection size:** 5 µL**Analysis****Samples:** *Standard solution* and *Sample solution*Measure the heights of the peaks due to castor oil and the *Internal standard solution*.

Calculate the percentage of castor oil in the portion of Aromatic Castor Oil taken:

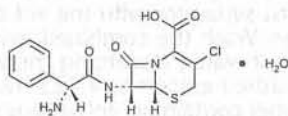
$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times 100$$

 $R_U$  = ratio of the heights of the peaks due to castor oil and the internal standard, *Sample solution* $R_S$  = ratio of the heights of the peaks due to castor oil and the internal standard, *Standard solution* $W_S$  = weight of Castor Oil taken to prepare the *Standard solution* (mg) $W_U$  = nominal weight of castor oil in the sample of Aromatic Castor Oil taken to prepare the *Sample solution* (mg)**Acceptance criteria:** NLT 95.0%**OTHER COMPONENTS**

- **ALCOHOL DETERMINATION, Method I** <611>: NMT 4.0% of  $C_2H_5OH$

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

**Cefaclor** $C_{15}H_{14}ClN_3O_4S \cdot H_2O$  385.825-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-chloro-8-oxo-, monohydrate, [6R-[6 $\alpha$ , -7 $\beta$ (R\*)]]-

(6R,7R)-7-[(R)-2-Amino-2-phenylacetamido]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate.

3-Chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxylic acid monohydrate [70356-03-5].

Anhydrous 367.81 [53994-73-3].

» Cefaclor has a potency of not less than 950 µg and not more than 1020 µg of  $C_{15}H_{14}ClN_3O_4S$  per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.**USP Reference standards** <11>—

USP Cefaclor RS

USP Cefaclor Delta-3 Isomer RS

**Identification**—**A:** *Infrared Absorption* <197K>.**B:** The retention time of the major peak for cefaclor in the chromatogram of the *Assay* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.**Crystallinity** <695>: meets the requirements.**pH** <791>: between 3.0 and 4.5, in an aqueous suspension containing 25 mg per mL.**Water Determination, Method I** <921>: between 3.0% and 6.5%.**Related compounds**—**Solvent**—Dissolve 2.4 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 2.5.**Blank solution**—Use the *Solvent*.**Solution A**—Dissolve 6.9 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 4.0.**Solution B**—Prepare a mixture of *Solution A* and acetonitrile (550:450), degassing for not more than 2 minutes.**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>). [NOTE—Reducing the acetonitrile content increases the retention time of cefaclor and increases the resolution between cefaclor, delta-3 isomer and cefaclor.]**Standard solution**—Dissolve an accurately weighed quantity of USP Cefaclor RS in *Solvent* to obtain a solution having a known concentration of about 0.05 mg per mL. Sonicate briefly, if necessary, to dissolve, and avoid heating. [NOTE—Use this solution on the day it is prepared.]**System suitability solution**—Dissolve a quantity of USP Cefaclor, Delta-3 Isomer RS in the *Standard solution* to obtain a solution having a concentration of about 0.05 mg per mL.**Test solutions**—Transfer about 50 mg of Cefaclor, accurately weighed, to each of two 10-mL volumetric flasks, dilute each with *Solvent* to volume, and mix. Sonicate briefly, if necessary, to dissolve, and avoid heating. [NOTE—Use these *Test solutions* within 2 hours when stored at room temperature or within 20 hours when stored under refrigeration.]**Chromatographic system** (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1 mL per minute. The chromatogram is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	95	5	equilibration
0–30	95→75	5→25	linear gradient
30–45	75→0	25→100	linear gradient
45–55	0	100	isocratic
55–60	0→95	100→5	reset composition
60–70	95	5	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*; the retention time for the cefaclor peak is between 23 and 29 minutes; the resolution,  $R$ , between cefaclor, delta-3 isomer and cefaclor is not less than 2.0; and the tailing factor for the cefaclor peak is not more than 1.2. Chromatograph the *Blank solution* as directed for *Procedure*. Examine the chromatogram for any extraneous peaks, and disregard any cor-



responding peaks observed in the chromatogram of the *Test solutions*. [NOTE—Ensure that any extraneous peaks observed do not represent carryover from previous injections.]

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solutions* into the chromatograph, record the chromatograms, and measure all of the peak areas. Calculate the percentage of each cefaclor related compound in the portion of Cefaclor taken by the formula:

$$(CP/W)(r_i / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Cefaclor RS in the *Standard solution*; *P* is the designated potency, in  $\mu$ g per mg, of USP Cefaclor RS; *W* is the weight, in mg, of the portion of Cefaclor taken to prepare the respective *Test solution*; *r<sub>i</sub>* is the peak response of an individual related compound in the chromatogram obtained from the *Test solution*; and *r<sub>s</sub>* is the peak response for the cefaclor peak in the chromatogram of the *Standard solution*. Determine the mean values for each cefaclor related compound: not more than 0.5% of any individual cefaclor related compound is found, and not more than 2.0% of total cefaclor related compounds is found. In an acceptable determination, the difference between duplicate determinations of total cefaclor related compounds is not more than 0.2% absolute, or the variation from the mean of the two values is not more than 10%, whichever is greater.

#### Assay—

**Mobile phase**—Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of water and 10 mL of triethylamine. Adjust with phosphoric acid to a pH of  $2.5 \pm 0.1$ , add 220 mL of methanol, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 15 mg of USP Cefaclor RS, accurately weighed, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Sonicate briefly, if necessary, to achieve dissolution, and avoid heating the solution. [NOTE—Use this *Standard preparation* within 8 hours if stored at room temperature, or within 20 hours if stored under refrigeration.]

**Assay preparation**—Transfer about 15 mg of Cefaclor, accurately weighed, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Sonicate briefly, if necessary, to achieve dissolution, and avoid heating the solution. [NOTE—Use this *Assay preparation* within 8 hours if stored at room temperature, or within 20 hours if stored under refrigeration.]

**Resolution solution**—Prepare a solution in *Mobile phase* containing about 0.3 mg of cefaclor and 0.3 mg of USP Cefaclor, Delta-3 Isomer RS per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 265-nm detector and a 4.6-mm  $\times$  25-cm column containing 5- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times for cefaclor and cefaclor, delta-3 isomer are about 0.8 and 1.0, the resolution, *R*, between the cefaclor peak and the cefaclor, delta-3 isomer peak is not less than 2.5, the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the potency, in  $\mu$ g

per mg, of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) in each mg of the Cefaclor taken by the formula:

$$(W_s / W_u)(P)(r_u / r_s)$$

in which *W<sub>s</sub>* and *W<sub>u</sub>* are the weights, in mg, of USP Cefaclor RS and of Cefaclor taken to prepare the *Standard preparation* and the *Assay preparation*, respectively; *P* is the designated potency, in  $\mu$ g of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) per mg, of USP Cefaclor RS; and *r<sub>u</sub>* and *r<sub>s</sub>* are the peak responses of the cefaclor peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefaclor Capsules

» Cefaclor Capsules contain the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{15}H_{14}ClN_3O_4S$ .

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Cefaclor RS

USP Cefaclor Delta-3 Isomer RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

#### Dissolution (711)—

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) dissolved from UV absorbances at the wavelength of maximum absorbances at about 264 nm of filtered portions of the solution under test, suitably diluted with water, in comparison with a *Standard solution* having a known concentration of USP Cefaclor RS in the same medium.

**Tolerances**—Not less than 80% (Q) of the labeled amount of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water Determination, Method I** (921): not more than 8.0%.

#### Related compounds—

**Diluent**—Dissolve 2.4 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 2.5.

**Blank solution**—Use the *Diluent*.

**Solution A**—Dissolve 6.9 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 4.0.

**Solution B**—Prepare a mixture of *Solution A* and acetonitrile (55:45), degassing for no longer than 2 minutes.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). [NOTE—Reducing the acetonitrile content increases the retention time of cefaclor and increases the resolution between the delta-3 isomer and cefaclor.]

**Standard solution**—Dissolve an accurately weighed quantity of USP Cefaclor RS in *Diluent* to obtain a solution having a known concentration of about 0.05 mg per mL of cefaclor. Sonicate briefly, if necessary, to dissolve, and avoid heating. [NOTE—Use this solution on the day it is prepared.]



**System suitability solution**—Dissolve a quantity of USP Cefaclor, Delta-3 Isomer RS in the *Standard solution* to obtain a solution having a known concentration of about 0.05 mg per mL of the delta-3 isomer.

**Test solution**—Remove as completely as possible the contents of not fewer than 20 Capsules, and mix. Transfer an accurately weighed portion of the combined contents, equivalent to about 50 mg of cefaclor, to a 10-mL volumetric flask. Dissolve in *Diluent*, using brief sonication, if necessary, to achieve dissolution. Avoid heating. Dilute with *Diluent* to volume, mix, and filter. This solution has a nominal concentration of 5 mg per mL based on the label claim. [NOTE—Use this *Test solution* within 3 hours if stored at room temperature, or within 20 hours when stored under refrigeration.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	95	5	equilibration
0–30	95→75	5→25	linear gradient
30–45	75→0	25→100	linear gradient
45–55	0	100	isocratic
55–60	0→95	100→5	reset composition
60–70	95	5	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: identify the peaks by their relative retention times, which are about 0.85 and 1.0 for the delta-3 isomer and cefaclor, respectively; the resolution,  $R$ , between the delta-3 isomer and cefaclor is not less than 2.0; and the tailing factor for the cefaclor peak is not more than 1.2. Chromatograph the *Blank solution* as directed for *Procedure*. Examine the chromatogram for any extraneous peaks, and disregard any corresponding peaks observed in the chromatogram of the *Test solution*.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses for all the peaks. Calculate the percentage of each related compound in the portion of Capsules taken by the formula:

$$100(P)(C_s / C_u)(r_i / r_s)$$

in which  $P$  is the potency, in mg of cefaclor, per mg of USP Cefaclor RS;  $C_s$  is the concentration, in mg per mL, of USP Cefaclor RS in the *Standard solution*;  $C_u$  is the nominal concentration, in mg per mL, of cefaclor in the *Test solution*;  $r_i$  is the peak response of an individual related compound in the chromatogram obtained from the *Test solution*; and  $r_s$  is the peak response for the cefaclor peak in the chromatogram of the *Standard solution*. The reporting level for impurities is 0.1%. Not more than 0.5% of any individual related compound is found; and the sum of all related compounds is not more than 2.0%.

#### Assay—

**Mobile phase**—Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 780 mL of water and 10 mL of triethylamine. Adjust with phosphoric acid to a pH of  $2.5 \pm 0.1$ , add 220 mL of methanol, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cefaclor RS in *Mobile phase* to obtain a solution having a known concentration of about 0.3 mg per mL of cefaclor. Sonicate briefly, if necessary, to achieve dissolution, and avoid heating the solution. [NOTE—Use this

*Standard preparation* within 8 hours if stored at room temperature, or within 20 hours if stored under refrigeration.]

**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules, and weigh accurately. Mix the combined contents, and transfer an accurately weighed portion of the powder, equivalent to about 75 mg of cefaclor, to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Sonicate if necessary to ensure complete dissolution of the cefaclor. Filter to obtain the clear *Assay preparation*. The nominal concentration of this solution is 0.3 mg per mL of cefaclor based on the label claim.

**System suitability solution**—Dissolve accurately weighed quantities of USP Cefaclor RS and USP Cefaclor Delta-3 Isomer RS in *Mobile phase* to obtain a solution having a known concentration of about 0.3 mg each of cefaclor and the delta-3 isomer per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 265-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the responses as directed for *Procedure*: identify the peaks by their relative retention times, which are about 0.8 and 1.0 for cefaclor and the delta-3 isomer, respectively; the resolution,  $R$ , between the cefaclor peak and the delta-3 isomer peak is not less than 2.5; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percent label claim of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) in the portion of Capsules taken by the formula:

$$(P)(C_s / C_u)(r_u / r_s)(100)$$

in which  $P$  is the potency, in mg, of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) per mg, of USP Cefaclor RS;  $C_s$  is the concentration, in mg per mL, of USP Cefaclor RS in the *Standard preparation*;  $C_u$  is the nominal concentration, in mg per mL, of cefaclor in the *Assay preparation*; and  $r_u$  and  $r_s$  are the peak responses of the cefaclor peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefaclor for Oral Suspension

» Cefaclor for Oral Suspension is a dry mixture of Cefaclor and one or more suitable buffers, colors, diluents, and flavors. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{15}H_{14}ClN_3O_4S$ .

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Cefaclor RS

USP Cefaclor Delta-3 Isomer RS

**Identification**—The retention time of the major peak for cefaclor in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

#### Uniformity of dosage units (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.



**Deliverable volume** (698): meets the requirements.

**pH** (791): between 2.5 and 5.0, in the suspension constituted as directed in the labeling.

**Water Determination, Method I** (921): not more than 2.0%.

**Related compounds—**

*Solvent, Blank solution, Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, and Chromatographic system*—Proceed as directed for Related compounds under Cefaclor.

**Test solution**—Constitute Cefaclor for Oral Suspension as directed in the labeling. Transfer an accurately measured portion of Cefaclor for Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 50 mg of cefaclor, to a 10-mL volumetric flask. Dissolve in *Solvent*, using brief sonication, if necessary, to achieve dissolution. Avoid heating. Dilute with *Solvent* to volume, mix, and filter. Use this *Test solution* within 3 hours if stored at room temperature, or within 20 hours when stored under refrigeration.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses for all the peaks. Calculate the mg of each related compound in the portion of Cefaclor for Oral Suspension taken by the formula:

$$0.01 CP(r_i / r_s)$$

in which the terms are as defined for *Related compounds* under Cefaclor. Not more than 1.0% of any individual cefaclor-related compound is found; and the sum of all cefaclor-related compounds found is not more than 3.0%, not including the contribution of any peak that gives a result of less than 0.1%.

**Assay—**

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under Cefaclor.

**Assay preparation**—Constitute Cefaclor for Oral Suspension as directed in the labeling. Transfer an accurately measured portion of the resulting suspension, freshly mixed and free from air bubbles, dilute quantitatively with *Mobile phase* to obtain a final solution containing about 0.3 mg of cefaclor per mL. Sonicate if necessary to ensure complete dissolution of the cefaclor. Filter to obtain the clear *Assay preparation*.

**Procedure**—Proceed as directed in the Assay under Cefaclor. Calculate the quantity, in mg, of  $C_{15}H_{14}ClN_3O_4S$  in the portion of the constituted Cefaclor for Oral Suspension taken by the formula:

$$V_U (W_S / 50)(P/1000)(r_U / r_S)$$

in which  $V_U$  is the final volume, in mL, of the *Assay preparation*, and the other terms are as defined therein.

## Cefaclor Chewable Tablets

» Cefaclor Chewable Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ).

**Packaging and storage**—Preserve in tight containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—The product label and product labeling indicate that the Chewable Tablets must be chewed or crushed before administration.

**USP Reference standards (11)—**

USP Cefaclor RS

USP Cefaclor Delta-3 Isomer RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

**Dissolution (711)—**

*Medium*: water; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of cefaclor dissolved by employing UV absorption at the wavelength of maximum absorbance at about 264 nm on filtered portions of the solution under test, suitably diluted with *Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Cefaclor RS in the same *Medium*. Calculate the amount of cefaclor dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times D \times LC}$$

in which  $A_U$  and  $A_S$  are the absorbances obtained from the solution under test and the *Standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage;  $D$  is the dilution factor of the solution under test; and  $LC$  is the Tablet label claim, in mg.

**Tolerances**—Not less than 80% (Q) of the labeled amount of cefaclor is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water Determination, Method I** (921): not more than 5.0%.

**Related compounds—**

*Solvent, Blank solution, Solution A, Solution B, Mobile phase, Standard solution, System suitability solution, and Chromatographic system*—Proceed as directed for Related compounds under Cefaclor.

**Test solution**—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the composite, equivalent to about 50 mg of cefaclor, to a 10-mL volumetric flask. Dissolve in *Solvent*, using brief sonication, if necessary, to dissolve. Avoid heating. Dilute with *Solvent* to volume, mix, and filter. [NOTE—Use this *Test solution* within 3 hours if stored at room temperature, or within 20 hours when stored under refrigeration.]

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses for all the peaks. Calculate the quantity, in mg, of each related compound in the portion of Chewable Tablets taken by the formula:

$$0.01 CP(r_i / r_s)$$

in which the terms are as defined for *Related compounds* under Cefaclor. Not more than 1.0% of any individual cefaclor related compound is found; and the sum of all cefaclor related compounds found is not more than 3.0%, not including the contribution of any peak that gives a result of less than 0.1%.

**Assay—**

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under Cefaclor.

**Assay preparation**—Weigh and finely powder not fewer than 20 Chewable Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of



cefaclor, to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Sonicate, if necessary, to dissolve the cefaclor. Filter to obtain a clear solution.

**Procedure**—Proceed as directed in the *Assay* under *Cefaclor*. Calculate the quantity, in mg, of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) in the portion of Chewable Tablets taken by the formula:

$$5W_s(P/1000)(r_u/r_s)$$

in which the terms are as defined therein.

## Cefaclor Extended-Release Tablets

» Cefaclor Extended-Release Tablets contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

### USP Reference standards (11)—

USP Cefaclor RS

USP Cefaclor Delta-3 Isomer RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

### Dissolution (711)—

*Medium*: 0.1 N hydrochloric acid; 900 mL.

*Apparatus 1* (10-mesh basket): 100 rpm.

*Times*: 30, 60, and 240 minutes.

**Procedure**—Quantitatively dilute filtered portions of the solution under test with 0.1 N hydrochloric acid to obtain a test solution having a concentration of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) estimated to be about 25 µg per mL. Determine the amount of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) dissolved by employing UV absorption at the wavelength of maximum absorbance at about 265 nm, in comparison with a Standard solution having a similar, known concentration of USP Cefaclor RS in the same *Medium*.

**Tolerances**—The percentages of the labeled amount of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) dissolved at the times specified conform to *Acceptance Table 2*.

Time (minutes)	Amount dissolved
30	between 5% and 30%
60	between 20% and 50%
240	not less than 80%

**Uniformity of dosage units** (905): meet the requirements.

**Water Determination, Method I** (921): not more than 7.0%.

### Related compounds—

*Solvent*, *Blank solution*, *Solution A*, *Solution B*, *Mobile phase*, *Standard solution*, *System suitability solution*, and *Chromatographic system*—Proceed as directed for *Related compounds* under *Cefaclor*.

**Test solution**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the composite, equivalent to about 50 mg of cefaclor, to a 10-mL volumetric flask. Dissolve in *Solvent*, using brief sonication, if necessary, to achieve dissolution. Avoid heating.

Dilute with *Solvent* to volume, mix, and filter. Use this *Test solution* within 3 hours if stored at room temperature, or within 20 hours when stored under refrigeration.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area responses for all the peaks. Calculate the mg of each related compound in the portion of Tablets taken by the formula:

$$0.01CP(r_i/r_s)$$

in which the terms are as defined for *Related compounds* under *Cefaclor*. Not more than 0.6% of any individual cefaclor-related compound is found; and the sum of all cefaclor-related compounds found is not more than 2.0%, not including the contribution of any peak that gives a result of less than 0.1%.

### Assay—

*Mobile phase*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cefaclor*.

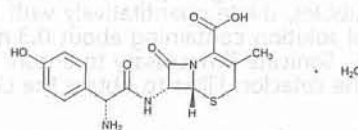
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of cefaclor, to a 250-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Sonicate, if necessary, to dissolve the cefaclor. Filter to obtain a clear solution.

**Procedure**—Proceed as directed in the *Assay* under *Cefaclor*. Calculate the quantity, in mg, of cefaclor ( $C_{15}H_{14}ClN_3O_4S$ ) in the portion of Tablets taken by the formula:

$$5W_s(P/1000)(r_u/r_s)$$

in which the terms are as defined therein.

## Cefadroxil



$C_{16}H_{17}N_3O_5S \cdot H_2O$	381.40
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[amino(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-, monohydrate, [6R-[6α,7β(R*)]]-;	
(6R,7R)-7-[(R)-2-Amino-2-(p-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate [66592-87-8].	
Hemihydrate [119922-85-9].	372.40
Anhydrous [50370-12-2].	363.39

### DEFINITION

Cefadroxil has a potency equivalent to NLT 950 µg/mg and NMT 1050 µg/mg of cefadroxil ( $C_{16}H_{17}N_3O_5S$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.



**ASSAY****• PROCEDURE**

**Buffer:** 2.7 g/L of monobasic potassium phosphate

**Mobile phase:** Acetonitrile and *Buffer* (4:96)

**Standard solution:** 50 µg/mL of USP Cefadroxil RS in *Mobile phase* prepared as follows. Transfer a suitable quantity of USP Cefadroxil RS to a suitable volumetric flask, dissolve in *Mobile phase* using 50% of the final volume, sonicate to dissolve, and dilute with *Mobile phase* to volume.

**Sample solution:** 50 µg/mL of Cefadroxil in *Mobile phase* prepared as follows. Transfer a suitable quantity of Cefadroxil to a suitable volumetric flask, dissolve in *Mobile phase* using 50% of the final volume, sonicate to dissolve, and dilute with *Mobile phase* to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 0.73%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of cefadroxil ( $C_{16}H_{17}N_3O_5S$ ) in the portion of Cefadroxil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cefadroxil RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Cefadroxil in the *Sample solution* (µg/mL)

$P$  = potency of cefadroxil in USP Cefadroxil RS (µg/mg)

**Acceptance criteria:** 950–1050 µg/mg on the anhydrous basis

**IMPURITIES****• ORGANIC IMPURITIES**

**Solution A:** 50 mg/mL of sodium hydroxide

**Solution B:** 4 g/L of monobasic sodium phosphate dihydrate adjusted with *Solution A* to a pH of 5.0

**Solution C:** Acetonitrile and *Solution B* (1:1)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution B (%)	Solution C (%)
0	100	0
35	85	15
50	40	60
60	0	100
61	100	0
70	100	0

**Diluent:** 3.5 g/L of monobasic potassium phosphate and 4.6 g/L of dibasic sodium phosphate anhydrous

**System suitability stock solution 1:** 0.5 mg/mL of USP Cefadroxil Related Compound D RS in *Diluent*. Sonicate as needed to dissolve.

**System suitability stock solution 2:** 0.5 mg/mL of USP Cefadroxil Related Compound I RS in *Diluent*. Sonicate as needed to dissolve.

**System suitability solution:** 10 µg/mL of cefadroxil related compound D from *System suitability stock solution 1*, 10 µg/mL of cefadroxil related compound I from *System suitability stock solution 2*, and 1 mg/mL of USP Cefadroxil System Suitability Mixture RS in *Solution B*. Store the sample in the refrigerator, and discard after 14 h.

**Standard solution:** 10 µg/mL of USP Cefadroxil RS in *Solution B*. Store the sample in the refrigerator, and discard after 14 h.

**Sample solution:** 1 mg/mL of Cefadroxil in *Solution B*. Store the sample in the refrigerator, and discard after 14 h.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Autosampler temperature:** 6°

**Injection volume:** 20 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between cefadroxil related compound D and cefadroxil related compound I, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cefadroxil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of cefadroxil from the *Standard solution*

$C_S$  = concentration of the USP Cefadroxil RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cefadroxil in the *Sample solution* (mg/mL)

$P$  = potency of cefadroxil in USP Cefadroxil RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** See *Table 2*. The reporting threshold is 0.03%.



Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amoxicillin related compound I <sup>a</sup>	0.16	0.5
Cefadroxil R-sulfoxide <sup>b</sup>	0.22	0.15
Cefadroxil S-sulfoxide <sup>c</sup>	0.41	0.15
Cefadroxil related compound B <sup>d</sup>	0.49	0.5
Dimethylformamide <sup>e</sup>	0.55	—
Cefadroxil related compound D <sup>f</sup>	0.71	0.5
Cefadroxil related compound I <sup>g</sup>	0.80	0.15
Diketopiperazine derivative <sup>h</sup>	0.87	0.5
Cefadroxil	1.0	—
N-Phenylglycyl delta-3 cefadroxil <sup>i</sup>	1.4	0.15
Cefadroxil ethyl homologue <sup>j</sup>	1.5	0.15
N-Phenylglycyl cefadroxil <sup>k</sup>	1.8	0.5
3-Hydroxy-4-methylthiophenone <sup>l</sup>	2.0	0.5
N-Ethoxycarbonyl 7-aminodesacetoxycephalosporanic acid <sup>m</sup>	2.1	0.15
O-Ethoxycarbonyl cefadroxil <sup>n</sup>	2.3	0.3
Cefadroxil dimer <sup>o</sup>	2.4	0.15
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

<sup>a</sup> D-Hydroxyphenylglycine; (R)-2-Amino-2-(4-hydroxyphenyl)acetic acid.

<sup>b</sup> (6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5R-oxide.

<sup>c</sup> (6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5S-oxide.

<sup>d</sup> 7-Aminodesacetoxycephalosporanic acid; (6R,7R)-7-Amino-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>e</sup> This impurity is listed here for information only. It is not to be reported as an impurity in this test.

<sup>f</sup> L-Cefadroxil; (6R,7R)-7-[(S)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>g</sup> Delta-3 cefadroxil; (6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.

<sup>h</sup> 3-(Aminomethylene)-6-(4-hydroxyphenyl)piperazine-2,5-dione.

<sup>i</sup> (6R,7R)-7-[(2R)-2-[2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.

<sup>j</sup> (6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-ethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>k</sup> (6R,7R)-7-[(2R)-2-[2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>l</sup> 3-Hydroxy-4-methylthiophen-2(5H)-one.

<sup>m</sup> (6R,7R)-7-(Ethoxycarbonylamino)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>n</sup> (6R,7R)-7-[(R)-2-Amino-2-[4-[(ethoxycarbonyl)oxy]phenyl]acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>o</sup> (6R,7R)-7-[(R)-2-[(6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamido]-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

- **DIMETHYLANILINE (223):** Meets the requirements

#### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation (781S)**  
Sample solution: 10 mg/mL in water  
Acceptance criteria: +165.0° to +178.0°
- **CRYSTALLINITY (695):** Meets the requirements
- **pH (791)**  
Sample solution: 50 mg/mL suspension in water  
Acceptance criteria: 4.0–6.0
- **WATER DETERMINATION, Method I (921):** 4.2%–6.0%, except where it is labeled as being in the hemihydrate form it is between 2.4% and 4.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The hemihydrate form is so labeled.
- **USP REFERENCE STANDARDS (11)**  
USP Cefadroxil RS  
USP Cefadroxil Related Compound D RS  
(6R,7R)-7-[(S)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.  
C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>S 363.39  
USP Cefadroxil Related Compound I RS  
(6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.  
C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>S 363.39  
USP Cefadroxil System Suitability Mixture RS  
This is a mixture of cefadroxil and O-ethoxycarbonyl cefadroxil [(6R,7R)-7-[(R)-2-Amino-2-[4-[(ethoxycarbonyl)oxy]phenyl]acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid].  
C<sub>19</sub>H<sub>21</sub>N<sub>3</sub>O<sub>7</sub>S 435.45

### Cefadroxil Capsules

#### DEFINITION

Cefadroxil Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cefadroxil (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>S).

#### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with 10 N potassium hydroxide to a pH of 5.0.

**Mobile phase:** Acetonitrile and *Buffer* (40:960)

**Standard solution:** 1.06 mg/mL of USP Cefadroxil RS in *Buffer*. This solution contains the equivalent of 1 mg/mL of cefadroxil. Use this solution on the day prepared.

**Sample solution:** Remove the contents of NLT 10 Capsules as completely as possible, and weigh. Transfer a portion of the powder, nominally equivalent to 200 mg of cefadroxil, to a 200-mL volumetric flask. Dilute with *Buffer* to volume, and stir by mechanical means for 5 min. Use this solution on the day prepared.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.2

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of cefadroxil (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>S) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*



- $C_s$  = concentration of USP Cefadroxil RS in the Standard solution (mg/mL)  
 $C_u$  = nominal concentration of cefadroxil in the Sample solution (mg/mL)  
 $P$  = potency of cefadroxil in USP Cefadroxil RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
 Acceptance criteria: 90.0%–120.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: USP Cefadroxil RS in Medium at a known concentration similar to that in the Sample solution

Sample solution: Sample per Dissolution (711). Suitably dilute with Medium, if necessary, and filter.

Analysis: Determine the amount of cefadroxil ( $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_5\text{S}$ ) dissolved from UV absorbances at 263 nm of the Sample solution in comparison to the Standard solution.

Tolerances: NLT 80% (Q) of the labeled amount of cefadroxil ( $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_5\text{S}$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

Solution A: 50 mg/mL of sodium hydroxide

Solution B: 4 g/L of monobasic sodium phosphate dihydrate in water, adjusted with Solution A to a pH of 5.2

Solution C: Acetonitrile and Solution B (1:1)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
35	85	15
50	40	60
60	0	100
61	100	0
70	100	0

Diluent: 3.5 g/L of monobasic potassium phosphate and 4.6 g/L of dibasic sodium phosphate anhydrous  
 System suitability stock solution 1: 0.5 mg/mL of USP Cefadroxil Related Compound D RS in Diluent. Sonicate as needed to dissolve.

System suitability stock solution 2: 0.5 mg/mL of USP Cefadroxil Related Compound I RS in Diluent. Sonicate as needed to dissolve.

System suitability solution: 10  $\mu\text{g}/\text{mL}$  of cefadroxil related compound D from System suitability stock solution 1, 10  $\mu\text{g}/\text{mL}$  of cefadroxil related compound I from System suitability stock solution 2, and 1 mg/mL of USP Cefadroxil System Suitability Mixture RS in Solution B. Store refrigerated, and discard after 14 h.

Standard solution: 10  $\mu\text{g}/\text{mL}$  of USP Cefadroxil RS in Solution B

Sample solution: Nominally, 1 mg/mL of cefadroxil in Solution B prepared as follows. Transfer the finely powdered contents of NLT 10 Capsules to a suitable volumetric flask, and add 50% of the final volume of Solution B. Sonicate with intermittent shaking. Cool to room temperature, dilute with Solution B to final volume, and mix. Centrifuge a portion of this solution, and filter the supernatant solution. Store refrigerated, and discard after 14 h.

## Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm  $\times$  25-cm; 5- $\mu\text{m}$  packing L1

Flow rate: 1 mL/min

Injection volume: 20  $\mu\text{L}$

Autosampler temperature: 6°

## System suitability

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for cefadroxil related compound D, cefadroxil related compound I, and cefadroxil are about 0.71, 0.80, and 1.0, respectively.]

## Suitability requirements

Resolution: NLT 1.5 between cefadroxil related compound D and cefadroxil related compound I, System suitability solution

Relative standard deviation: NMT 5.0%, Standard solution

## Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times (F_1/F_2) \times 100$$

$r_u$  = peak response of each impurity from the Sample solution

$r_s$  = peak response of cefadroxil from the Standard solution

$C_s$  = concentration of USP Cefadroxil RS in the Standard solution (mg/mL)

$C_u$  = nominal concentration of cefadroxil in the Sample solution (mg/mL)

$P$  = potency of cefadroxil in USP Cefadroxil RS ( $\mu\text{g}/\text{mg}$ )

$F_1$  = conversion factor, 0.001 mg/ $\mu\text{g}$

$F_2$  = relative response factor (see Table 2)

Acceptance criteria: See Table 2. The reporting threshold is 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound I <sup>a</sup>	0.16	1.3	0.5
Cefadroxil related compound B <sup>b</sup>	0.52	0.63	0.5
Cefadroxil related compound D <sup>c,d</sup>	0.71	—	—

<sup>a</sup> D-Hydroxyphenylglycine; (R)-2-Amino-2-(4-hydroxyphenyl)acetic acid.

<sup>b</sup> 7-Aminodesacetoxycephalosporanic acid; (6R,7R)-7-Amino-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>c</sup> L-Cefadroxil; (6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>d</sup> Process impurities that are controlled in the drug substance are not to be reported, are not included in total impurities, and are listed here for information only.

<sup>e</sup> 3-(Aminomethylene)-6-(4-hydroxyphenyl)piperazine-2,5-dione.

<sup>f</sup> (6R,7R)-7-[(2R)-2-[2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.

<sup>g</sup> (6R,7R)-7-[(2R)-2-[2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>h</sup> 3-Hydroxy-4-methylthiophen-2(5H)-one.

<sup>i</sup> (6R,7R)-7-(Ethoxycarbonylamino)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>j</sup> (6R,7R)-7-[(R)-2-Amino-2-[4-[(ethoxycarbonyl)oxy]phenyl]acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.



Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Diketopiperazine derivative <sup>a</sup>	0.89	1.3	0.5
Cefadroxil	1.0	—	—
N-Phenylglycyl delta-3 cefadroxil <sup>f</sup>	1.4	1.5	0.15
N-Phenylglycyl cefadroxil <sup>g,d</sup>	1.8	—	—
3-Hydroxy-4-methylthiophenone <sup>h</sup>	1.9	0.40	0.5
N-Ethoxycarbonyl 7-aminodesacetoxycephalosporanic acid <sup>i,d</sup>	2.2	—	—
O-Ethoxycarbonyl cefadroxil <sup>i,d</sup>	2.4	—	—
Any individual, unspecified impurity	—	—	0.2
Total impurities	—	—	2.0

<sup>a</sup> D-Hydroxyphenylglycine; (R)-2-Amino-2-(4-hydroxyphenyl)acetic acid.<sup>b</sup> 7-Aminodesacetoxycephalosporanic acid; (6R,7R)-7-Amino-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>c</sup> L-Cefadroxil; (6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>d</sup> Process impurities that are controlled in the drug substance are not to be reported, are not included in total impurities, and are listed here for information only.<sup>e</sup> 3-(Aminomethylene)-6-(4-hydroxyphenyl)piperazine-2,5-dione.<sup>f</sup> (6R,7R)-7-[(2R)-2-[2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.<sup>g</sup> (6R,7R)-7-[(2R)-2-[2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>h</sup> 3-Hydroxy-4-methylthiophen-2(SH)-one.<sup>i</sup> (6R,7R)-7-(Ethoxycarbonylamino)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>j</sup> (6R,7R)-7-[(R)-2-Amino-2-(4-[(ethoxycarbonyl)oxy]phenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.**SPECIFIC TESTS**

- **WATER DETERMINATION**, Method I (921): NMT 7.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **LABELING**: Capsules prepared using the hemihydrate form of Cefadroxil are so labeled.
- **USP REFERENCE STANDARDS (11)**
  - USP Cefadroxil RS
  - USP Cefadroxil Related Compound D RS  
(6R,7R)-7-[(S)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.  
 $C_{16}H_{17}N_3O_5S$  363.39
  - USP Cefadroxil Related Compound I RS  
(6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.  
 $C_{16}H_{17}N_3O_5S$  363.39
  - USP Cefadroxil System Suitability Mixture RS  
This is a mixture of cefadroxil and O-ethoxycarbonyl cefadroxil [(6R,7R)-7-[(R)-2-Amino-2-(4-[(ethoxycarbonyl)oxy]phenyl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid].  
 $C_{19}H_{21}N_3O_7S$  435.45

**Cefadroxil for Oral Suspension****DEFINITION**

Cefadroxil for Oral Suspension is a dry mixture of Cefadroxil and one or more suitable buffers, colors, diluents, and flavors. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of  $C_{16}H_{17}N_3O_5S$ .

**IDENTIFICATION**• **THIN-LAYER CHROMATOGRAPHY**

**Standard solution:** 2 mg/mL of USP Cefadroxil RS

**Sample solution:** Constitute 1 container of Cefadroxil for Oral Suspension as directed in the labeling. Dilute a portion of the resulting suspension with water to a concentration of 2 mg/mL. Pass through a suitable filter.

**Chromatographic system**

(See Chromatography (621), Thin-Layer Chromatography.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of binder-free silica gel

**Application volume:** 20  $\mu$ L

**Pre-developing solvent system:** *n*-Hexane and tetradecane (95:5)

**Solution A:** 1 in 15 solution of ninhydrin in acetone

**Developing solvent system:** 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and Solution A (60: 40: 1.5)

**Spray reagent:** 1 in 500 solution of ninhydrin in dehydrated alcohol. Protect this solution from light.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Place the thin-layer chromatographic plate in a chamber containing the *Pre-developing solvent system* and allow the solvent front to move the length of the plate. Remove the plate from the chamber and allow the solvent to evaporate. Apply the *Sample solution* and *Standard solution* to the plate, allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Spray the plate with the *Spray reagent*, dry for 10 min at 110°, and examine the chromatogram.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

**ASSAY**• **PROCEDURE**

**Buffer:** 6.86 g/L of monobasic potassium phosphate.

Adjust with 10 N potassium hydroxide to a pH of 5.0.

**Mobile phase:** Acetonitrile and Buffer (40:960)

**Standard solution:** 1.06 mg/mL of USP Cefadroxil RS in Buffer. Use this solution on the day prepared. [NOTE—This solution contains the equivalent of 1 mg/mL of cefadroxil ( $C_{16}H_{17}N_3O_5S$ ).]

**Sample solution:** Constitute a container of Cefadroxil for Oral Suspension as directed in the labeling. Dilute a portion of the resulting suspension with Buffer to prepare a solution containing nominally 1 mg/mL. Pass through a suitable filter of 0.8- $\mu$ m or finer pore size, and use the filtrate. Use this solution on the day prepared.

**Chromatographic system**

(See Chromatography (621), System Suitability.)



Mode: LC

Detector: UV 230 nm

Column: 4-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection size: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor,  $k'$ : 2.0–3.5

Column efficiency: NLT 1800 theoretical plates

Tailing factor: NMT 2.2

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefadroxil ( $C_{16}H_{17}N_3O_5S$ ) in the portion of Cefadroxil for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of cefadroxil from the *Sample solution*

$r_S$  = peak response of cefadroxil from the *Standard solution*

$C_S$  = concentration of USP Cefadroxil RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefadroxil in the *Sample solution* (mg/mL)

$P$  = potency of cefadroxil in USP Cefadroxil RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 25 rpm

Time: 30 min

Standard solution: USP Cefadroxil RS in *Medium* at a known concentration

Sample solution: Transfer 5.0 mL of the constituted Cefadroxil for Oral Suspension (weighed) to the dissolution vessel.

Analysis: Determine the amount of cefadroxil dissolved by employing UV absorption at the wavelength of 263 nm on the *Sample solution* in comparison with the *Standard solution*.

Calculate the amount of cefadroxil dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/D) \times V$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$D$  = dilution factor

Tolerances: NLT 75% (Q) of the labeled amount of cefadroxil is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905)

For solid packaged in single-unit containers: Meets the requirements

### • DELIVERABLE VOLUME (698): Meets the requirements

## SPECIFIC TESTS

### • pH (791): 4.5–6.0, in the suspension constituted as directed in the labeling

### • WATER DETERMINATION, Method I (921): NMT 2.0%, except where it is labeled as containing 100 mg of

cefadroxil per mL after constitution, in which case the limit is NMT 3.0%

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
USP Cefadroxil RS

## Cefadroxil Tablets

### DEFINITION

Cefadroxil Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of cefadroxil ( $C_{16}H_{17}N_3O_5S$ ).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHY

Standard solution: 2 mg/mL of USP Cefadroxil RS

Sample solution: Nominally 2 mg/mL of cefadroxil from powdered Tablets dissolved in water and filtered

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Adsorbent: 0.25-mm layer of binder-free silica gel

Application volume: 20 µL

Pre-developing solvent solution: *n*-Hexane and tetradecane (95:5)

Solution A: 66.7-mg/mL solution of ninhydrin in acetone

Developing solvent system: 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and *Solution A* (60: 40: 1.5)

Spray reagent: 2-mg/mL solution of ninhydrin in dehydrated alcohol. Protect from light.

#### Analysis

Samples: *Standard solution* and *Sample solution*

Place the thin-layer chromatographic plate in a chamber containing the *Pre-developing solvent solution* to a depth of about 1 cm, and allow the solvent front to move the length of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. Apply the *Sample solution* and *Standard solution* to the plate, allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow to air-dry. Spray the plate with the *Spray reagent*, dry for 10 min at 110°, and examine the chromatogram.

Acceptance criteria: The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

## ASSAY

### • PROCEDURE

Buffer: 6.8 g/L of monobasic potassium phosphate in water, adjusted with 10 N potassium hydroxide to a pH of 5.0

Mobile phase: Acetonitrile and *Buffer* (40:960)

Standard solution: 1.06 mg/mL of USP Cefadroxil RS in *Buffer*. This solution contains nominally 1 mg/mL of cefadroxil. Use this solution on the day prepared.

Sample solution: Nominally 1 mg/mL of cefadroxil from finely powdered Tablets (NLT 10) in *Buffer*. Stir by mechanical means for 5 min. Use this solution on the day prepared.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4-mm × 25-cm; packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 µL

**System suitability**Sample: *Standard solution*

Suitability requirements

Capacity factor,  $k'$ : 2.0–3.5

Column efficiency: NLT 1800 theoretical plates

Tailing factor: NMT 2.2

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of cefadroxil ( $C_{16}H_{17}N_3O_5S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Cefadroxil RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of cefadroxil in the *Sample solution* (mg/mL) $P$  = potency of cefadroxil in USP Cefadroxil RS (µg/mg) $F$  = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

**PERFORMANCE TESTS**• **DISSOLUTION** (711)

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

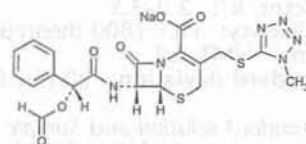
**Standard solution:** Prepare a solution having a known concentration of USP Cefadroxil RS in *Medium*.**Sample solution:** Sample per (711). Pass a portion of the solution under test through a suitable filter, and dilute with water if necessary.**Instrumental conditions**

Mode: UV

Analytical wavelength: 263 nm

**Analysis**Samples: *Standard solution* and *Sample solution*Tolerances: NLT 75% (Q) of the labeled amount of cefadroxil ( $C_{16}H_{17}N_3O_5S$ ) is dissolved.• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements**SPECIFIC TESTS**• **WATER DETERMINATION**, *Method I* (921): NMT 8.0%**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers.• **LABELING:** The Tablets prepared using the hemihydrate form of cefadroxil are so labeled.• **USP REFERENCE STANDARDS** (11)

USP Cefadroxil RS

**Cefamandole Nafate** $C_{19}H_{17}N_6NaO_5S_2$  512.495-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(formyloxy)phenylacetyl]amino]-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, monosodium salt, [6R-[6 $\alpha$ ,7 $\beta$  (R\*)]]-;

Sodium (6R,7R)-7-(R)-mandelamido-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate formate (ester) [42540-40-9].

**DEFINITION**Cefamandole Nafate has a potency equivalent to NLT 810 µg/mg and NMT 1000 µg/mg of cefamandole ( $C_{18}H_{18}N_6O_5S_2$ ), calculated on the anhydrous basis.**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE****Buffer:** 10% solution of triethylamine in water, adjusted with phosphoric acid to a pH of 2.5**Mobile phase:** Acetonitrile and *Buffer* (25:75)**Standard solution:** 0.5 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Use this solution immediately after it is prepared.**System suitability solution:** 0.05 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Heat at 60° for 30 min.**Sample solution:** 0.5 mg/mL of Cefamandole Nafate in *Mobile phase*. Use this solution immediately after it is prepared.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 20 µL

**System suitability**Samples: *Standard solution* and *System suitability solution*

Suitability requirements

**Resolution:** NLT 7.0 between the two main peaks, *System suitability solution***Relative standard deviation:** NMT 0.8% for the cefamandole peak, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*Calculate the concentration, in µg/mg, of cefamandole ( $C_{18}H_{18}N_6O_5S_2$ ) in the portion of Cefamandole Nafate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times P \times F$$

 $r_U$  = sum of the peak responses of cefamandole and cefamandole nafate from the *Sample solution*



- $r_s$  = sum of the peak responses of cefamandole and cefamandole nafate from the *Standard solution*  
 $C_s$  = concentration of USP Cefamandole Nafate RS in the *Standard solution* (mg/mL)  
 $C_u$  = concentration of the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of cefamandole, 462.50  
 $M_{r2}$  = molecular weight of cefamandole nafate, 512.49  
 $P$  = potency of cefamandole nafate in USP Cefamandole Nafate RS (mg/mg)  
 $F$  = conversion factor, 1000  $\mu\text{g}/\text{mg}$   
**Acceptance criteria:** 810–1000  $\mu\text{g}/\text{mg}$  on the anhydrous basis

### SPECIFIC TESTS

- PH (791)**  
 Sample solution: 100 mg/mL  
 Acceptance criteria: 3.5–7.0
- WATER DETERMINATION, Method I (921):** NMT 2.0%
- BACTERIAL ENDOTOXINS TEST (85):** NMT 0.15 USP Endotoxin Unit/mg of cefamandole, when the label states that Cefamandole Nafate is sterile or must be subjected to further processing during the preparation of injectable dosage forms
- STERILITY TESTS (71):** Meets the requirements for *Test for Sterility of the Product to Be Examined, Membrane Filtration* when the label states that Cefamandole Nafate is sterile

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers.
- LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- USP REFERENCE STANDARDS (11)**  
 USP Cefamandole Nafate RS  
 USP Endotoxin RS

## Cefamandole Nafate for Injection

### DEFINITION

Cefamandole Nafate for Injection is a sterile mixture of Cefamandole Nafate and one or more suitable buffers. It has a potency equivalent to NLT 810  $\mu\text{g}/\text{mg}$  and NMT 1000  $\mu\text{g}/\text{mg}$  of cefamandole ( $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_2$ ), calculated on the anhydrous and sodium carbonate-free basis. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of cefamandole ( $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_2$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Buffer:** 10% solution of triethylamine in water, adjusted with phosphoric acid to a pH of 2.5

**Mobile phase:** Acetonitrile and *Buffer* (25:75)

**Standard solution:** 0.5 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Use this solution immediately after it is prepared.

**System suitability solution:** 0.05 mg/mL of USP Cefamandole Nafate RS in *Mobile phase*. Heat at 60° for 30 min.

**Sample solution:** Equivalent to 0.5 mg/mL of cefamandole nafate from Cefamandole Nafate for Injection in *Mobile phase*. Use this solution immediately after it is prepared.

**Sample solution 1** (where the article is represented as being in a single-dose container): Equivalent to

0.5 mg/mL of cefamandole nafate from Cefamandole Nafate for Injection in *Mobile phase* prepared as follows. Constitute a container of Cefamandole Nafate for Injection in a volume of *Mobile phase* corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with *Mobile phase*.

**Sample solution 2** (where the label states the quantity of cefamandole in a given volume of constituted solution): Equivalent to 0.5 mg/mL of cefamandole nafate from Cefamandole Nafate for Injection in *Mobile phase* prepared as follows. Constitute Cefamandole Nafate for Injection in a volume of *Mobile phase* corresponding to the volume of solvent specified in the labeling. Dilute a volume of the constituted solution with *Mobile phase*.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu\text{m}$  packing L1

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu\text{L}$

### System suitability

**Samples:** *Standard solution* and *System suitability solution*

### Suitability requirements

**Resolution:** NLT 7.0 between the two main peaks, *System suitability solution*

**Relative standard deviation:** NMT 0.8% for the cefamandole peak, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of cefamandole ( $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_2$ ) in the portion of constituted solution taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times P \times 100$$

$r_u$  = sum of the peak responses of cefamandole and cefamandole nafate from *Sample solution 1* or *Sample solution 2*

$r_s$  = sum of the peak responses of cefamandole and cefamandole nafate from the *Standard solution*

$C_s$  = concentration of USP Cefamandole Nafate RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of cefamandole in *Sample solution 1* or in *Sample solution 2* (mg/mL)

$M_{r1}$  = molecular weight of cefamandole, 462.50

$M_{r2}$  = molecular weight of cefamandole nafate, 512.49

$P$  = potency of USP Cefamandole Nafate RS (mg/mg)

Calculate the potency, in  $\mu\text{g}/\text{mg}$ , of cefamandole ( $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_2$ ) in the portion of Cefamandole Nafate for Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times P \times F$$

$r_u$  = sum of the peak responses of cefamandole and cefamandole nafate from *Sample solution 1* or *Sample solution 2*

$r_s$  = sum of the peak responses of cefamandole and cefamandole nafate from the *Standard solution*

$C_s$  = concentration of USP Cefamandole Nafate RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of cefamandole in *Sample solution 1* or *Sample solution 2* (mg/mL)

$M_{r1}$  = molecular weight of cefamandole, 462.50

$M_{r2}$  = molecular weight of cefamandole nafate, 512.49



$P$  = potency of USP Cefamandole Nafate RS (mg/mg)

$F$  = conversion factor, 1000  $\mu\text{g}/\text{mg}$

**Acceptance criteria:** 810–1000  $\mu\text{g}/\text{mg}$  of  $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_2$ , calculated on the anhydrous and sodium carbonate-free basis; 90.0%–115.0% of the labeled amount of  $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_2$

## PERFORMANCE TESTS

### • UNIFORMITY OF DOSAGE UNITS (905)

**Procedure for content uniformity:** Perform the Assay on individual containers using *Sample solution 1* or *Sample solution 2*, or both, as appropriate.

**Acceptance criteria:** Meets the requirements

## SPECIFIC TESTS

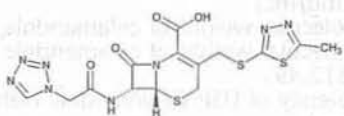
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.15 USP Endotoxin Unit/mg of cefamandole
- **STERILITY TESTS (71):** It meets the requirements for *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.
- **pH (791)**  
Sample solution: 100 mg/mL  
Acceptance criteria: 6.0–8.0, determined after 30 min
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **WATER DETERMINATION, Method I (921):** NMT 3.0%
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* (1).

## ADDITIONAL REQUIREMENTS

### Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).
- **USP REFERENCE STANDARDS (11)**  
USP Cefamandole Nafate RS  
USP Endotoxin RS

## Cefazolin



$\text{C}_{14}\text{H}_{14}\text{N}_8\text{O}_4\text{S}_3$  454.51  
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[[5-methyl-1,3,4-thiadiazol-2-yl]thio]methyl]-8-oxo-7-[[[1H-tetrazol-1-yl]acetyl]amino]-(6R-trans); (6R,7R)-3-[[[5-methyl-1,3,4-thiadiazol-2-yl]thio]methyl]-8-oxo-7-[[[2-[(1H-tetrazol-1-yl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [25953-19-9].

## DEFINITION

Cefazolin contains NLT 95.0% and NMT 103.0% of cefazolin ( $\text{C}_{14}\text{H}_{14}\text{N}_8\text{O}_4\text{S}_3$ ), calculated on the anhydrous basis.

## IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

## ASSAY

### • PROCEDURE

Protect all solutions containing cefazolin from light.

**Buffer A:** 0.9 g/L of anhydrous dibasic sodium phosphate and 1.3 g/L of citric acid monohydrate in water

**Buffer B:** 5.7 g/L of anhydrous dibasic sodium phosphate and 3.6 g/L of monobasic potassium phosphate in water

**Mobile phase:** Acetonitrile and Buffer A (10:90)

**Standard solution:** 50  $\mu\text{g}/\text{mL}$  of USP Cefazolin RS in Buffer B

**Sample solution:** 50  $\mu\text{g}/\text{mL}$  of Cefazolin in Buffer B

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu\text{m}$  packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu\text{L}$

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Relative standard deviation:** NMT 2.0%

**Tailing factor:** NMT 1.5

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cefazolin ( $\text{C}_{14}\text{H}_{14}\text{N}_8\text{O}_4\text{S}_3$ ) in the portion of Cefazolin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak response of cefazolin from the *Sample solution*

$r_S$  = peak response of cefazolin from the *Standard solution*

$C_S$  = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cefazolin in the *Sample solution* (mg/mL)

$P$  = potency of cefazolin in USP Cefazolin RS (mg/mg)

**Acceptance criteria:** 95.0%–103.0% on the anhydrous basis

## IMPURITIES

### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-Jan-2018)

### • ORGANIC IMPURITIES

Protect all solutions containing cefazolin from light.

**Solution A:** 6.8 g/L of monobasic potassium phosphate in water

**Solution B:** 6.8 g/L of monobasic potassium phosphate adjusted with 10% sodium hydroxide to a pH of 6.8 before final dilution with water

**Solution C:** Acetonitrile and *Solution A* (1:1)

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	98	2
7	98	2
15	85	15
30	80	20
35	80	20
45	50	50
50	50	50
55	98	2
65	98	2



**Blank:** Use *Solution B*.

**System suitability stock solution:** 2 mg/mL of USP Cefazolin RS in 0.05 M sodium hydroxide. Set the solution aside at room temperature for 5 min. [NOTE—The cefazolin epimer is formed upon treatment of cefazolin with sodium hydroxide.]

**System suitability solution:** *System suitability stock solution* and *Solution B* (1:24)

**Standard solution:** 25 µg/mL of USP Cefazolin RS in *Solution B*. Use this solution immediately after preparation.

**Sample solution:** 2.5 mg/mL of Cefazolin in *Solution B*. Use this solution immediately after preparation.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 and 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 8.0 between cefazolin and cefazolin epimer, 254 nm

#### Analysis

**Samples:** *Blank*, *Standard solution*, and *Sample solution*  
Calculate the percentage of tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin taken:

$$\text{Result} = (r_{U(210)} / r_{S(254)}) \times (C_S / C_U) \times (1 / F) \times 100$$

$r_{U(210)}$  = peak response of tetrazolylacetic acid or tetrazolylacetamide acetal at 210 nm from the *Sample solution*

$r_{S(254)}$  = peak response of cefazolin at 254 nm from the *Standard solution*

$C_S$  = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the Cefazolin in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

Calculate the percentage of each impurity other than tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin taken:

$$\text{Result} = (r_{U(254)} / r_{S(254)}) \times (C_S / C_U) \times (1 / F) \times 100$$

$r_{U(254)}$  = peak response of each impurity other than tetrazolylacetic acid or tetrazolylacetamide acetal at 254 nm from the *Sample solution*

$r_{S(254)}$  = peak response of cefazolin at 254 nm from the *Standard solution*

$C_S$  = concentration of USP Cefazolin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the Cefazolin in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*. Disregard peaks corresponding to those in the *Blank*.

**Table 2**

Name	Analytical Wave-length (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tetrazolylacetic acid <sup>a</sup>	210	0.07	0.40	1.0
Tetrazolylacetamide acetal <sup>b</sup>	210	0.08	0.33	1.0
Cefazolin open-ring lactone <sup>c,d</sup> or Cefazolin 3-hydroxymethyl <sup>e</sup>	254	0.20	1.0	0.5
Methylthiadiazole thiol <sup>f</sup>	254	0.23	0.91	1.0
7-Aminocephalosporanic acid <sup>g</sup>	254	0.42	1.1	1.0
Cefazolin 3-methyl analog <sup>h</sup>	254	0.44	0.87	1.0
Cefazolin lactone <sup>i</sup>	254	0.50	0.85	1.0
Cefazolin acetoxymethyl analog <sup>j</sup>	254	0.61	0.68	1.0
Cefazolin deacetylated <sup>k</sup>	254	0.68	1.2	1.0
Cefazolin acid isomers <sup>l</sup>	254	0.84	1.0	1.0
Cefazolin	254	1.0	—	—
Cefazolin epimer <sup>m</sup>	254	1.2	0.98	1.0
Cefazolin pivaloyl <sup>n</sup>	254	1.4	0.92	1.0

<sup>a</sup> 2-(1*H*-Tetrazol-1-yl)acetic acid.

<sup>b</sup> *N*-(2,2-Dihydroxyethyl)-2-(1*H*-tetrazol-1-yl)acetamide.

<sup>c</sup> The identification of this impurity is tentative. The names of the most likely compounds are listed in footnotes <sup>d</sup> and <sup>e</sup>.

<sup>d</sup> (R)-2-[2-(1*H*-Tetrazol-1-yl)acetamido]-2-[(R)-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*]1,3]thiazin-2-yl]acetic acid.

<sup>e</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> 5-Methyl-1,3,4-thiadiazole-2-thiol (MMTD).

<sup>g</sup> (6*R*,7*R*)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA).

<sup>h</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>i</sup> *N*-[(5*a*,6*R*)-1,7-Dioxo-1,3,4,5*a*,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*]1,3]thiazin-6-yl]-2-(1*H*-tetrazol-1-yl)acetamide.

<sup>j</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>k</sup> (6*R*,7*R*)-7-Amino-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>l</sup> Three isomers of this impurity may not be fully resolved by this method. The limit applies to the sum of the isomers, which are as follows:

Cefazolin open-ring delta-3: (2*R*)-2-[(R)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxymethyl)-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-2: (2*R*)-2-[(R)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxymethyl)-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,4-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-4: (2*R*)-2-[(R)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxymethyl)-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

<sup>m</sup> (6*R*,7*S*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>n</sup> (6*R*,7*R*)-3-[(5-Methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-7-pivalamido-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.



Table 2 (Continued)

Name	Analytical Wave-length (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	254	—	1.0	0.1
Total impurities	—	—	—	3.5

<sup>a</sup> 2-(1*H*-Tetrazol-1-yl)acetic acid.

<sup>b</sup> *N*-(2,2-Dihydroxyethyl)-2-(1*H*-tetrazol-1-yl)acetamide.

<sup>c</sup> The identification of this impurity is tentative. The names of the most likely compounds are listed in footnotes <sup>d</sup> and <sup>e</sup>.

<sup>d</sup> (6*R*)-2-[2-(1*H*-Tetrazol-1-yl)acetamido]-2-[(*R*)-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*][1,3]thiazin-2-yl]acetic acid.

<sup>e</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> 5-Methyl-1,3,4-thiadiazole-2-thiol (MMTD).

<sup>g</sup> (6*R*,7*R*)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA).

<sup>h</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>i</sup> *N*-[(5*aR*,6*R*)-1,7-Dioxo-1,3,4,5*a*,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*][1,3]thiazin-6-yl]-2-(1*H*-tetrazol-1-yl)acetamide.

<sup>j</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>k</sup> (6*R*,7*R*)-7-Amino-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>l</sup> Three isomers of this impurity may not be fully resolved by this method. The limit applies to the sum of the isomers, which are as follows:

Cefazolin open-ring delta-3: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxymethyl)-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-2: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxymethyl)-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,4-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-4: (2*R*)-2-[(*R*)-[2-(1*H*-Tetrazol-1-yl)acetamido](carboxymethyl)-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

<sup>m</sup> (6*R*,7*S*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>n</sup> (6*R*,7*R*)-3-[(5-Methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-7-pivalamido-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

## SPECIFIC TESTS

- **WATER DETERMINATION**, Method I (921): NMT 2.0%

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
USP Cefazolin RS

## Cefazolin Injection

» Cefazolin Injection is a sterile solution of Cefazolin and Sodium Bicarbonate in a diluent containing one or more suitable tonicity-adjusting agents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cefazolin (C<sub>14</sub>H<sub>14</sub>N<sub>8</sub>O<sub>4</sub>S<sub>3</sub>).

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

## USP Reference standards (11)—

USP Cefazolin RS

USP Endotoxin RS

**Identification**—The retention time of the major peak for cefazolin in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.15 USP Endotoxin Unit per mg of cefazolin.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.5 and 7.0.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

## Assay—

**pH 3.6 Buffer**—Dissolve 0.900 g of anhydrous dibasic sodium phosphate and 1.298 g of citric acid monohydrate in water to make 1000 mL.

**pH 7.0 Buffer**—Dissolve 5.68 g of anhydrous dibasic sodium phosphate and 3.63 g of monobasic potassium phosphate in water to make 1000 mL.

**Mobile phase**—Prepare a suitable mixture of pH 3.6 Buffer and acetonitrile (9:1). Pass through a membrane filter having a 10-μm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Transfer 750 mg of salicylic acid to a 100-mL volumetric flask, dissolve in 10 mL of methanol, dilute with pH 7.0 Buffer to volume, and mix.

**Standard preparation**—Transfer about 25 mg of USP Cefazolin RS, accurately weighed, to a 25-mL volumetric flask, dissolve in and dilute with pH 7.0 Buffer to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with pH 7.0 Buffer to volume, and mix.

**Assay preparation**—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the Injection, equivalent to about 50 mg of cefazolin, to a 50-mL volumetric flask, dilute with pH 7.0 Buffer to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with pH 7.0 Buffer to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm × 30-cm column that contains 10-μm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*. The relative retention times are about 0.7 for salicylic acid and 1.0 for cefazolin; the resolution, *R*, between the analyte and internal standard peaks is not less than 4.0; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cefazolin (C<sub>14</sub>H<sub>14</sub>N<sub>8</sub>O<sub>4</sub>S<sub>3</sub>) in each mL of Injection taken by the formula:

$$(1000C / V)(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Cefazolin RS, calculated on the anhydrous basis, in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of cefazolin to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Cefazolin for Injection

### DEFINITION

Cefazolin for Injection contains an amount of Cefazolin Sodium equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of ( $C_{14}H_{14}N_8O_4S_3$ ).

### IDENTIFICATION

#### A. ULTRAVIOLET ABSORPTION (197U)

Sample solution: 20 µg/mL in 0.1 M sodium bicarbonate

Acceptance criteria: Meets the requirements

#### B. The retention time of the major peak from the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

#### C. IDENTIFICATION TESTS—GENERAL, Sodium (191): Meets the requirements

### ASSAY

#### PROCEDURE

Buffer A: 0.9 g/L of anhydrous dibasic sodium phosphate and 1.298 g/L of citric acid monohydrate in water. The pH of Buffer A is 3.6.

Buffer B: 5.68 g/L of anhydrous dibasic sodium phosphate and 3.63 g/L of monobasic potassium phosphate in water. The pH of Buffer B is 7.0.

Mobile phase: Acetonitrile and Buffer A (10:90). Pass through a membrane filter having a 10-µm or finer pore size.

Internal standard solution: 7.5 mg/mL of salicylic acid in methanol and Buffer B (10:90) prepared as follows. Transfer a suitable portion of salicylic acid to a suitable volumetric flask, dissolve first in methanol using 10% of the final volume, dilute with Buffer B to volume, and mix.

Standard stock solution: 1 mg/mL of USP Cefazolin RS in Buffer B

Standard solution: Transfer 5.0 mL of the Standard stock solution to a 100-mL volumetric flask, add 5.0 mL of Internal standard solution, dilute with Buffer B to volume, and mix.

Sample stock solution 1 (where it is packaged for dispensing and is represented as being in a single-dose container): Nominally 1 mg/mL of cefazolin from Cefazolin for Injection prepared as follows. Constitute Cefazolin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with Buffer B.

Sample solution 1: Transfer 5.0 mL of the Sample stock solution 1 to a 100-mL volumetric flask, add 5.0 mL of Internal standard solution, dilute with Buffer B to volume, and mix.

Sample stock solution 2 (where the label states the quantity of cefazolin in a given volume of constituted solution): Nominally 1 mg/mL of cefazolin from Cefazolin for Injection prepared as follows. Constitute Cefazolin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Dilute an aliquot of the constituted solution with Buffer B.

Sample solution 2: Transfer 5.0 mL of the Sample stock solution 2 to a 100-mL volumetric flask, add 5.0 mL of Internal standard solution, dilute with Buffer B to volume, and mix.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.0-mm × 30-cm; 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

#### System suitability

Sample: Standard solution

[NOTE—The relative retention times for salicylic acid and cefazolin are about 0.7 and 1.0, respectively.]

#### Suitability requirements

Resolution: NLT 4.0 between the analyte and internal standard peaks

Column efficiency: NLT 1500 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: Standard solution, Sample solution 1, or Sample solution 2

Calculate the percentage of the labeled amount of cefazolin ( $C_{14}H_{14}N_8O_4S_3$ ) in the container and in the volume of constituted solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times 100$$

$R_U$  = peak response ratio of cefazolin to the internal standard of the Sample solution

$R_S$  = peak response ratio of cefazolin to the internal standard of the Standard solution

$C_S$  = concentration of USP Cefazolin RS, calculated on the anhydrous basis, in the Standard solution (mg/mL)

$C_U$  = nominal concentration of cefazolin in the Sample solution (mg/mL)

$P$  = potency of cefazolin in USP Cefazolin RS (mg/mg)

Acceptance criteria: 90.0%–115.0%. Where the test for Uniformity of Dosage Units (905) has been performed using the Analysis for content uniformity, use the average of these determinations as the Assay value.

### PERFORMANCE TESTS

#### UNIFORMITY OF DOSAGE UNITS (905)

##### Procedure for content uniformity

Perform the Assay on individual containers using Sample solution 1 or Sample solution 2, or both, as appropriate.

Acceptance criteria: Meets the requirements

### SPECIFIC TESTS

• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for Injections and Implanted Drug Products (1), Specific Tests, Completeness and clarity of solutions.

• **OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 55 mg/mL in 0.1 M sodium bicarbonate

Acceptance criteria:  $-10^\circ$  to  $-24^\circ$

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.15 USP Endotoxin Unit/mg of cefazolin

• **STERILITY TESTS (71):** It meets the requirements when tested as directed in Test for Sterility of the Product to Be Examined, Membrane Filtration.

• **pH (791)**

Sample solution: 100 mg/mL of cefazolin

Acceptance criteria: 4.0–6.0

• **WATER DETERMINATION, Method I (921):** NMT 6.0%

• **PARTICULATE MATTER IN INJECTION (788):** Meets the requirements for small-volume injections

• **OTHER REQUIREMENTS:** Meets the requirements in Labeling (7), Labels and Labeling for Injectable Products

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve as described in Packaging and Storage Requirements (659), Injection Packaging.



• **USP REFERENCE STANDARDS** (11)

USP Cefazolin RS  
USP Endotoxin RS

## Cefazolin Ophthalmic Solution

### DEFINITION

Cefazolin Ophthalmic Solution contains an amount of cefazolin sodium equivalent to NLT 29.7 mg and NMT 36.3 mg of cefazolin ( $C_{14}H_{14}N_8O_4S_3$ ) in 10.0 mL of Ophthalmic Solution.

Use Cefazolin Sodium or Cefazolin for Injection that contains the designated amount of cefazolin, and prepare the Ophthalmic Solution as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Cefazolin Sodium	35 mg
Thimerosal	0.2 mg
Sodium Chloride Injection (0.9%), a sufficient quantity to make	10.0 mL

Dissolve Cefazolin Sodium and Thimerosal in Sodium Chloride Injection (0.9%), and dilute quantitatively, and stepwise if necessary, with Sodium Chloride Injection (0.9%) to obtain a solution containing 3.5 mg/mL of Cefazolin Sodium and 0.02 mg/mL of Thimerosal. Filter a 10.0-mL portion of the resulting solution to produce a clear and sterile Ophthalmic Solution. If Cefazolin for Injection is used, prepare the Ophthalmic Solution as follows. Dissolve Thimerosal in Sodium Chloride Injection (0.9%), and dilute quantitatively, and stepwise if necessary, with Sodium Chloride Injection (0.9%) to obtain a solution containing 0.3 mg/mL of Thimerosal. Add 9.8 mL of the resulting solution to a vial of Cefazolin for Injection, containing 500 mg of cefazolin, and mix to obtain a stock solution. Transfer 3.3 mL of the stock solution to a 50-mL volumetric flask, dilute with Sodium Chloride Injection (0.9%) to volume, and mix. Filter a 10.0-mL portion of the resulting solution to produce a clear and sterile Ophthalmic Solution.

### ASSAY

#### • PROCEDURE

**Buffer A:** 0.900 g/L of anhydrous dibasic sodium phosphate and 1.298 g/L of citric acid monohydrate; this solution should have a pH of 3.6.

**Buffer B:** 5.68 g/L of anhydrous dibasic sodium phosphate and 3.63 g/L monobasic potassium phosphate; this solution should have a pH of 7.0.

**Solution A:** Acetonitrile and Buffer A (10:90). Pass through a filter having a pore size of 5- $\mu$ m or finer, and degas.

**Solution B:** Acetonitrile and Buffer A (80:20). Pass through a filter having a pore size of 5- $\mu$ m or finer, and degas.

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	0	100
25	100	0

**Standard solution:** 0.32 mg/mL of USP Cefazolin RS in Buffer B. Maintain at 4° before injection. Use low-actinic volumetric glassware.

**Sample solution:** Transfer 1.0 mL of Ophthalmic Solution to a 10-mL low-actinic volumetric flask, dilute with

Buffer B to volume, and mix. Maintain at 4° before injection.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 273 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu$ m packing L1

**Column temperature:** 25°

**Flow rate:** 2 mL/min

**Injection volume:** 10  $\mu$ L

### System suitability

**Sample:** Standard solution

### Suitability requirements

**Column efficiency:** NLT 1500 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0% for replicate injections

### Analysis

**Samples:** Standard solution and Sample solution

Calculate the quantity, in mg, of cefazolin ( $C_{14}H_{14}N_8O_4S_3$ ) in 10 mL of Ophthalmic Solution:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Cefazolin RS in the Standard solution (mg/mL)

$D$  = dilution factor, 10

$V$  = final volume of Ophthalmic Solution, 10 mL

**Acceptance criteria:** 29.7–36.3 mg

### SPECIFIC TESTS

#### • STERILITY

(See *Pharmaceutical Compounding—Nonsterile Preparations* (795), *General Guidelines for Assigning Beyond-Use Dates*.)

**Acceptance criteria:** Meets the requirements

#### • pH (791): 4.5–6.0

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in tight, sterile ophthalmic containers. Store in a refrigerator.

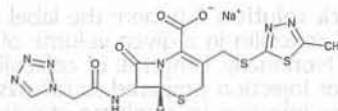
• **LABELING:** Label it to state that it is intended for use in the eye, and is not to be used if a precipitate is present.

• **BEYOND-USE DATE:** NMT 5 days after the date on which it was compounded

#### • USP REFERENCE STANDARDS (11)

USP Cefazolin RS

## Cefazolin Sodium



$C_{14}H_{13}N_8NaO_4S_3$  476.49

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[[5-methyl-1,3,4-thiadiazol-2-yl]thio]methyl]-8-oxo-7-[[[(1H-tetrazol-1-yl)acetyl]amino]-, monosodium salt (6R-trans-);

Monosodium (6R,7R)-3-[[[5-methyl-1,3,4-thiadiazol-2-yl]thio]methyl]-8-oxo-7-[[[(1H-tetrazol-1-yl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate; [27164-46-1].

### DEFINITION

Cefazolin Sodium has a potency equivalent to NLT 89.1% and NMT 110.1% of cefazolin sodium ( $C_{14}H_{13}N_8NaO_4S_3$ ), calculated on the anhydrous basis.



**IDENTIFICATION****A. INFRARED ABSORPTION (197K)**

Sample: 150 mg

Solution A: Acetone and water (9:1)

Analysis: Dissolve the Sample in 5 mL of water, add 0.5 mL of 2 N glacial acetic acid, swirl, and allow to stand for 10 min in an ice bath. Filter the precipitate, and rinse with 1–2 mL of water. Dissolve in Solution A, evaporate the solvent almost to dryness, and dry in an oven at 60° for 30 min. Prepare a standard specimen with USP Cefazolin RS treated similarly.

Acceptance criteria: Meets the requirements

**B.** The retention time of the major peak for cefazolin in the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.**C. IDENTIFICATION TESTS—GENERAL, Sodium (191):** Meets the requirements**ASSAY****PROCEDURE**

Protect all solutions containing cefazolin from light.

Buffer A: 0.9 g/L of anhydrous dibasic sodium phosphate and 1.3 g/L of citric acid monohydrate in water

Buffer B: 5.7 g/L of anhydrous dibasic sodium phosphate and 3.6 g/L of monobasic potassium phosphate in water

Mobile phase: Acetonitrile and Buffer A (1:9). Pass through a suitable filter.

Standard solution: 50 µg/mL of USP Cefazolin RS in Buffer B

Sample solution: 50 µg/mL of Cefazolin Sodium in Buffer B

**Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 2 mL/min

Injection volume: 10 µL

**System suitability**

Sample: Standard solution

**Suitability requirements**

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

**Analysis**

Samples: Standard solution and Sample solution

Calculate the percentage of cefazolin sodium ( $C_{14}H_{13}N_3NaO_4S_3$ ) in the portion of Cefazolin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times P \times 100$$

$r_U$  = peak response of cefazolin from the Sample solution

$r_S$  = peak response of cefazolin from the Standard solution

$C_S$  = concentration of USP Cefazolin RS in the Standard solution (mg/mL)

$C_U$  = concentration of Cefazolin Sodium in the Sample solution (mg/mL)

$M_{r1}$  = molecular weight of cefazolin sodium, 476.49

$M_{r2}$  = molecular weight of cefazolin, 454.51

$P$  = potency of USP Cefazolin RS (mg/mg)

Acceptance criteria: 89.1%–110.1% on the anhydrous basis

**IMPURITIES****ORGANIC IMPURITIES**

Protect all solutions containing cefazolin from light.

Solution A: 6.8 g/L of monobasic potassium phosphate

Solution B: 6.8 g/L of monobasic potassium phosphate adjusted with 10% sodium hydroxide to a pH of 6.8 before final dilution

Solution C: Acetonitrile and Solution A (1:1)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	98	2
7	98	2
15	85	15
30	80	20
35	80	20
45	50	50
50	50	50
55	98	2
65	98	2

Blank: Solution B

System suitability stock solution: 2 mg/mL of USP Cefazolin RS in 0.05 M sodium hydroxide. Set the solution aside at room temperature for 5 min. [NOTE—The cefazolin epimer is formed upon treatment of cefazolin with sodium hydroxide.]

System suitability solution: System suitability stock solution and Solution B (1:24)

Standard solution: 25 µg/mL of USP Cefazolin RS in Solution B. Use this solution promptly after preparation.

Sample solution: 2.5 mg/mL of Cefazolin Sodium in Solution B. Use this solution promptly after preparation.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 210 and 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

**System suitability**

Sample: System suitability solution

**Suitability requirements**

Resolution: NLT 8.0 between cefazolin and cefazolin epimer, 254 nm

**Analysis**

Samples: Blank, Standard solution, and Sample solution  
Calculate the percentage of tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin Sodium taken:

$$\text{Result} = (r_{U(210)}/r_{S(254)}) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{U(210)}$  = peak response of tetrazolylacetic acid or tetrazolylacetamide acetal at 210 nm from the Sample solution

$r_{S(254)}$  = peak response of cefazolin at 254 nm from the Standard solution

$C_S$  = concentration of USP Cefazolin RS in the Standard solution (mg/mL)

$C_U$  = concentration of Cefazolin Sodium in the Sample solution (mg/mL)

$F$  = relative response factor (see Table 2)

Calculate the percentage of each impurity other than tetrazolylacetic acid and tetrazolylacetamide acetal in the portion of Cefazolin Sodium taken:

$$\text{Result} = (r_{U(254)}/r_{S(254)}) \times (C_S/C_U) \times (1/F) \times 100$$

$r_{U(254)}$  = peak response of each impurity other than tetrazolylacetic acid and tetrazolylacetamide acetal at 254 nm from the Sample solution

$r_{S(254)}$  = peak response of cefazolin at 254 nm from the Standard solution

$C_S$  = concentration of USP Cefazolin RS in the Standard solution (mg/mL)



$C_U$  = concentration of Cefazolin Sodium in the Sample solution (mg/mL)

$F$  = relative response factor (see Table 2)

Acceptance criteria: See Table 2. Disregard peaks corresponding to those in the Blank.

Table 2

Name	Analytical Wave-length (nm)	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Tetrazolylacetic acid <sup>a</sup>	210	0.07	0.40	1.0
Tetrazolylacetamide acetate <sup>b</sup>	210	0.08	0.33	1.0
Cefazolin open-ring lactone <sup>c,d</sup> or Cefazolin 3-hydroxymethyl <sup>c,e</sup>	254	0.20	1.0	0.5
Methylthiadiazole thiol <sup>f</sup>	254	0.23	0.91	1.0
7-Aminocephalosporanic acid <sup>g</sup>	254	0.42	1.1	1.0
Cefazolin 3-methyl analog <sup>h</sup>	254	0.44	0.87	1.0
Cefazolin lactone <sup>i</sup>	254	0.50	0.85	1.0
Cefazolin acetoxy analog <sup>j</sup>	254	0.61	0.68	1.0
Cefazolin deacylated <sup>k</sup>	254	0.68	1.2	1.0
Cefazoloic acid isomers <sup>l</sup>	254	0.84	1.0	1.0
Cefazolin	254	1.0	—	—
Cefazolin epimer <sup>m</sup>	254	1.2	0.98	1.0
Cefazolin pivaloyl <sup>n</sup>	254	1.4	0.92	1.0
Any individual unspecified impurity	254	—	1.0	0.1
Total impurities	—	—	—	3.5

<sup>a</sup> 2-(1*H*-Tetrazol-1-yl)acetic acid.

<sup>b</sup> *N*-(2,2-Dihydroxyethyl)-2-(1*H*-tetrazol-1-yl)acetamide.

<sup>c</sup> The identification of this impurity is tentative. The names of the most likely compounds are listed in footnotes <sup>d</sup> and <sup>e</sup>.

<sup>d</sup> (R)-2-[2-(1*H*-Tetrazol-1-yl)acetamido]-2-[(R)-7-oxo-2,4,5,7-tetrahydro-1*H*-furo[3,4-*d*]1,3]thiazin-2-yl]acetic acid.

<sup>e</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> 5-Methyl-1,3,4-thiadiazole-2-thiol (MMTD).

<sup>g</sup> (6*R*,7*R*)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (7-ACA).

<sup>h</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>i</sup> *N*-[(5*aR*,6*R*)-1,7-Dioxo-1,3,4,5*a*,6,7-hexahydroazeto[2,1-*b*]furo[3,4-*d*]1,3]thiazin-6-yl]-2-(1*H*-tetrazol-1-yl)acetamide.

<sup>j</sup> (6*R*,7*R*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-(acetoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>k</sup> (6*R*,7*R*)-7-Amino-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>l</sup> Three isomers of this impurity may not be fully resolved by this method. The limit applies to the sum of the isomers, which are as follows:

Cefazolin open-ring delta-3: (2*R*)-2-[(R)-2-(1*H*-Tetrazol-1-yl)acetamido](carboxy)methyl]-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-2: (2*R*)-2-[(R)-2-(1*H*-Tetrazol-1-yl)acetamido](carboxy)methyl]-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-3,4-dihydro-2*H*-1,3-thiazine-4-carboxylic acid. Cefazolin open-ring delta-4: (2*R*)-2-[(R)-2-(1*H*-Tetrazol-1-yl)acetamido](carboxy)methyl]-5-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-5,6-dihydro-2*H*-1,3-thiazine-4-carboxylic acid.

<sup>m</sup> (6*R*,7*S*)-7-[2-(1*H*-Tetrazol-1-yl)acetamido]-3-[(5-methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>n</sup> (6*R*,7*R*)-3-[(5-Methyl-1,3,4-thiadiazol-2-ylthio)methyl]-8-oxo-7-pivalamido-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

## SPECIFIC TESTS

### • OPTICAL ROTATION, Specific Rotation (781S)

Sample solution: 55 mg/mL in 0.1 M sodium bicarbonate

Acceptance criteria:  $-10^{\circ}$  to  $-24^{\circ}$

### • PH (791)

Sample solution: 100 mg/mL of cefazolin

Acceptance criteria: 4.0–6.0

### • WATER DETERMINATION, Method I (921): NMT 6.0%

• **STERILITY TESTS (71):** Where the label states that Cefazolin Sodium is sterile, it meets the requirements when tested as directed for Test for Sterility of the Product to Be Examined, Membrane Filtration.

• **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Cefazolin Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Unit/mg of cefazolin.

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

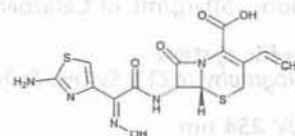
• **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

### • USP REFERENCE STANDARDS (11)

USP Cefazolin RS

USP Endotoxin RS

## Cefdinir



$C_{14}H_{13}N_5O_5S_2$  395.41

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(2-amino-4-thiazolyl)(hydroxyimino)acetyl]amino]-3-ethenyl-8-oxo-, [6*R*-(6*a*,7*B*(*Z*))]-; (-)-(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7*Z*-(*Z*)-oxime [91832-40-5].

## DEFINITION

Cefdinir contains NLT 940 µg/mg and NMT 1030 µg/mg of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ), calculated on the anhydrous basis.

## IDENTIFICATION

### • A. INFRARED ABSORPTION (197M)

• **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

## ASSAY

### • PROCEDURE

**Solution A:** 14.2 g/L of anhydrous dibasic sodium phosphate

**Solution B:** 13.6 g/L of monobasic potassium phosphate

**Solution C:** Dilute tetramethylammonium hydroxide (10%) with water to obtain a 0.1% solution. Adjust with 10% phosphoric acid to a pH of 5.5.

**Solution D:** 37.2 mg/mL of edetate disodium

**Buffer:** Combine appropriate amounts of Solution A and Solution B (about 2:1) to obtain a solution with a pH of 7.0.

**Mobile phase:** Acetonitrile, methanol, Solution C, and Solution D (300:200:4500:2)

**System suitability solution:** 0.2 mg/mL of USP Cefdinir RS and 0.5 mg/mL of USP Cefdinir Related Compound A RS in Buffer



Standard solution: 0.2 mg/mL of USP Cefdinir RS in Buffer

Sample solution: 0.2 mg/mL of Cefdinir in Buffer

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection size: 5 μL

#### System suitability

Samples: System suitability solution and Standard solution. USP Cefdinir Related Compound A RS should produce four peaks.

Tailing factor: NMT 1.5 for cefdinir, System suitability solution

Resolution: NLT 1.2 between the second peak of cefdinir related compound A and cefdinir, System suitability solution

Relative standard deviation: NMT 1.0%, Standard solution

#### Analysis

Samples: Standard solution and Sample solution

Calculate the quantity, in μg/mg, of cefdinir

(C<sub>14</sub>H<sub>13</sub>N<sub>3</sub>O<sub>5</sub>S<sub>2</sub>) in the portion of Cefdinir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of the Standard solution (mg/mL)

$C_U$  = concentration of the Sample solution (mg/mL)

$P$  = purity of USP Cefdinir RS (μg/mg)

Acceptance criteria: 940–1030 μg/mg on the anhydrous basis

#### IMPURITIES

- RESIDUE ON IGNITION (281): NMT 0.20%

#### Delete the following:

- HEAVY METALS, Method II (231): 10 ppm (Official 1-Jan-2018)

#### • ORGANIC IMPURITIES

Solution A, Solution B, Solution C, Solution D, and

Buffer: Prepare as directed in the Assay.

Solution E: To 1000 mL of Solution C add 0.4 mL of Solution D.

Solution F: Acetonitrile, methanol, Solution C, and Solution D (300:200:500:0.4)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution E (%)	Solution F (%)
0	95	5
2	95	5

Table 1 (Continued)

Time (min)	Solution E (%)	Solution F (%)
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

System suitability solution 1: 15 μg/mL of cefdinir from the Sample solution, diluted with Solution C

System suitability solution 2: 1.5 μg/mL of cefdinir from System suitability solution 1, diluted with Solution C

System suitability solution 3: 1.5 mg/mL of USP Cefdinir RS and 0.1 mg/mL of USP Cefdinir Related Compound A RS, dissolved initially in Buffer corresponding to 15% of the final volume, and diluted with Solution C to volume

Sample stock solution: 10 mg/mL of Cefdinir in Buffer

Sample solution: 1.5 mg/mL of cefdinir from the Sample stock solution, in Solution C. [NOTE—Prepare fresh immediately before use.]

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection size: 10 μL

#### System suitability

Samples: System suitability solution 1, System suitability solution 2, and System suitability solution 3. USP Cefdinir Related Compound A RS should produce four peaks.

#### Suitability requirements

Response ratio: The response of cefdinir from System suitability solution 2 is between 7% and 13% of that from System suitability solution 1.

Resolution: NLT 1.5 between cefdinir and the third peak of USP Cefdinir Related Compound A RS, System suitability solution 3

Relative standard deviation: NMT 2.0% for cefdinir, System suitability solution 3

#### Analysis

Sample: Sample solution. Record the chromatogram for at least 1.8 times the retention time of the cefdinir peak.

Calculate the percentage of each impurity in the portion of Cefdinir taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the Sample solution

$r_T$  = sum of all the peak responses from the Sample solution

Acceptance criteria: See Table 2.



Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Thiazolylacetyl glycine oxime <sup>a</sup>	0.10	0.5
Thiazolylacetyl glycine oxime acetal <sup>b</sup>	0.12	0.5
3-Methyl cefdinir <sup>c</sup>	0.74	0.7
Cefdinir related compound A (cefdinir open ring lactone a) <sup>d,e</sup>	0.85	0.7
Cefdinir related compound A (cefdinir open ring lactone b) <sup>d,e</sup>	0.93	
Cefdinir related compound A (cefdinir open ring lactone c) <sup>d,e</sup>	1.11	
Cefdinir related compound A (cefdinir open ring lactone d) <sup>d,e</sup>	1.14	
Cefdinir lactone <sup>f</sup>	1.22	0.5
Cefdinir isoxazole analog <sup>g</sup>	1.36	0.5
E-Cefdinir <sup>h</sup>	1.51	0.7
Cefdinir decarboxy open ring lactone a <sup>i</sup>	1.61	0.5
Cefdinir decarboxy open ring lactone b <sup>i</sup>	1.64	
Any other individual, unidentified impurity	—	0.2
Total impurities	—	3.0

<sup>a</sup> 1N-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.

<sup>b</sup> (Z)-2-(2-Aminothiazol-4-yl)-N-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.

<sup>c</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>d</sup> 2(R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2R,5R)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid.

<sup>e</sup> Cefdinir related compound A is a mixture of 4 isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported. The limit for the sum of the 4 isomers is 0.7%.

<sup>f</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(3R,5aR,6R)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]acetamide.

<sup>g</sup> (6R,7R)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>h</sup> (6R,7R)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>i</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(2R,5R)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]methyl]acetamide.

<sup>j</sup> Cefdinir decarboxy open ring lactone is a mixture of 2 isomers labeled cefdinir decarboxy open ring lactones a and b. The sum of the values is reported. The limit for sum of the 2 isomers is 0.5%.

## SPECIFIC TESTS

### • OPTICAL ROTATION, Specific Rotation (781S)

Sample solution: 10 mg/mL in Buffer, as obtained in the Assay

Acceptance criteria:  $-61^{\circ}$  to  $-67^{\circ}$  at  $20^{\circ}$

### • WATER DETERMINATION, Method I (921):

NMT 2.0% for anhydrous; 4.0%–8.5% for hydrated forms. For this monograph, the term “hydrated forms” refers to several known forms of Cefdinir. Use a mixture of formamide and methanol (2:1) as the solvent.

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE:

Preserve in tight, light-resistant containers.

### • USP REFERENCE STANDARDS (11)

USP Cefdinir RS

USP Cefdinir Related Compound A RS

(2R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2R,5R)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid (three other stereoisomers are also present in this RS).

$C_{14}H_{13}N_5O_6S_2$  413.43

## Cefdinir Capsules

### DEFINITION

Cefdinir Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of cefdinir ( $C_{14}H_{13}N_5O_6S_2$ ).

### IDENTIFICATION

#### • A. ULTRAVIOLET ABSORPTION (197U)

Buffer: Prepare as directed in the Assay.

Standard solution: 10  $\mu$ g/mL of USP Cefdinir RS in Buffer

Sample solution: Equivalent to 10  $\mu$ g/mL of cefdinir from Capsules in Buffer. Filter before use.

Cell size: 1 cm

Blank: Use the Buffer.

Acceptance criteria: Sample solution maxima and minima occur at the same wavelengths as those in the Standard solution.

#### • B.

The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

Buffer: 10.7 g/L of dibasic sodium phosphate and 3.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid or sodium hydroxide to a pH of  $7.0 \pm 0.05$  before final dilution.

Solution A: 7 g/L of citric acid monohydrate. Adjust with phosphoric acid to a pH of  $2.0 \pm 0.05$ .

Mobile phase: Methanol, tetrahydrofuran, and Solution A (11:28:1000)

System suitability solution: 50  $\mu$ g/mL of USP Cefdinir RS and 175  $\mu$ g/mL of *m*-hydroxybenzoic acid in Buffer

Standard solution: 50  $\mu$ g/mL of USP Cefdinir RS in Buffer

Sample solution: Equivalent to 50  $\mu$ g/mL of cefdinir, from Capsule contents (NLT 20), in Buffer

#### Chromatographic system

(See Chromatography (621), System Suitability.)  
Mode: LC

Detector: UV 254 nm

Column: 3.9-mm  $\times$  15-cm; 4- $\mu$ m packing L1

Flow rate: 1.4 mL/min

Injection volume: 15  $\mu$ L

#### System suitability

Samples: System suitability solution and Standard solution



**Suitability requirements**

**Resolution:** Greater than 3.0 between cefdinir and *m*-hydroxybenzoic acid, *System suitability solution*

**Tailing factor:** NMT 2.0 for cefdinir, *System suitability solution*

**Relative standard deviation:** NMT 1.0% for cefdinir, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cefdinir from the *Sample solution*

$r_S$  = peak response of cefdinir from the *Standard solution*

$C_S$  = concentration of the *Standard solution* ( $\mu\text{g/mL}$ )

$C_U$  = nominal concentration of cefdinir in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS****• DISSOLUTION (711)**

**Medium:** 50 mM phosphate buffer, pH 6.8; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Detector:** UV 290 nm

**Cell length:** 0.1-cm flow cell

**Standard solution:** 0.33 mg/mL of USP Cefdinir RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu\text{m}$  pore size. Dilute with *Medium* to a concentration of about 0.33 mg/mL of cefdinir.

**Blank:** Dissolve 1 empty Capsule in 100 mL of *Medium*, and dilute to 900 mL. Filter if necessary.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (1/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$D$  = dilution factor for the *Sample solution* (mL/mL)

$L$  = label claim (mg/Capsule)

**Tolerances:** NLT 80% (Q) of the labeled amount of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ) is dissolved.

**• UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**IMPURITIES****• ORGANIC IMPURITIES**

**Solution A:** 14.2 g/L of anhydrous dibasic sodium phosphate

**Solution B:** 13.6 g/L of monobasic potassium phosphate

**Buffer:** Combine appropriate amounts of *Solution A* and *Solution B* (about 2:1) to obtain a solution with a pH of  $7.0 \pm 0.1$ .

**Solution C:** Dilute tetramethylammonium hydroxide (10% aqueous) with water to obtain a 0.1% solution. Adjust with dilute phosphoric acid (1 in 10) to a pH of  $5.5 \pm 0.1$ .

**Solution D:** 37.2 mg/mL of edetate disodium

**Solution E:** To 1000 mL of *Solution C* add 0.4 mL of *Solution D*.

**Solution F:** Acetonitrile, methanol, *Solution C*, and *Solution D* (150:100:250:0.2)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution E (%)	Solution F (%)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

**System suitability stock solution 1:** 40  $\mu\text{g/mL}$  of USP Cefdinir Related Compound A RS in *Solution C*

**System suitability stock solution 2:** 40  $\mu\text{g/mL}$  of USP Cefdinir Related Compound B RS in *Solution C*

**System suitability solution:** Transfer 37.5 mg of USP Cefdinir RS to a 25-mL volumetric flask. Add about 10 mL of *Buffer*. Add 5.0 mL each of *System suitability stock solution 1* and *System suitability stock solution 2*, and dilute with *Solution C* to volume.

**Standard stock solution:** 750  $\mu\text{g/mL}$  of USP Cefdinir RS in *Buffer*

**Standard solution:** 15  $\mu\text{g/mL}$  of USP Cefdinir RS, from the *Standard stock solution*, in *Solution C*

**Sample solution:** Transfer an equivalent to 300 mg of cefdinir from Capsule contents (NLT 20) to a 200-mL volumetric flask. Dissolve in 30 mL of *Buffer*, and dilute with *Solution C* to volume to obtain a solution with a nominal concentration of about 1.5 mg/mL of cefdinir.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu\text{m}$  packing L1

**Temperatures**

**Autosampler:** 4°

**Column:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu\text{L}$

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between cefdinir and the third peak of USP Cefdinir Related Compound A RS, *System suitability solution*

**Tailing factor:** NMT 1.5 for cefdinir related compound B, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the cefdinir peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (100/F)$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefdinir in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*. The reporting threshold is 0.1%.



Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Thiazolylacetyl glycine oxime <sup>a</sup>	0.10	1.0	0.5
Thiazolylacetyl glycine oxime acetal <sup>b</sup>	0.13	1.0	0.5
Cefdinir sulfoxide <sup>c</sup>	0.36	1.0	0.2
Cefdinir thiazine analog <sup>d</sup>	0.46	0.68	0.7
3-Methyl cefdinir <sup>e</sup>	0.75	1.0	0.7
Cefdinir impurity 1 <sup>f</sup>	0.77	1.0	0.3
Cefdinir related compound A (cefdinir open ring lactone a) <sup>g,h</sup>	0.85	0.65	2.5
Cefdinir related compound A (cefdinir open ring lactone b) <sup>g,h</sup>	0.94	0.65	
Cefdinir related compound A (cefdinir open ring lactone c) <sup>g,h</sup>	1.11	0.65	
Cefdinir related compound A (cefdinir open ring lactone d) <sup>g,h</sup>	1.14	0.65	
7S-Cefdinir <sup>i</sup>	1.18	1.0	0.2
Cefdinir lactone <sup>j</sup>	1.23	1.0	1.0
Cefdinir related compound B <sup>k</sup>	1.28	1.0	0.2
Cefdinir isoxazole analog <sup>l</sup>	1.37	0.72	0.5
Cefdinir impurity 2 <sup>l</sup>	1.44	1.0	0.5
Cefdinir glyoxalic analog <sup>m</sup>	1.49	1.0	0.2
E-Cefdinir <sup>n</sup>	1.51	1.0	1.2

<sup>a</sup> N-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.<sup>b</sup> (Z)-2-(2-Aminothiazol-4-yl)-N-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.<sup>c</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>d</sup> (R,Z)-2-[(R)-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido](carboxymethyl)-5-ethylidene-5,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.<sup>e</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>f</sup> Cefdinir impurity 1, cefdinir impurity 2, and cefdinir impurity 3 are unidentified impurities.<sup>g</sup> Cefdinir related compound A is a mixture of four isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported. The limit for the sum of the four isomers is 2.5%.<sup>h</sup> 2(R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2RS,5RS)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid.<sup>i</sup> (6R,7S)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>j</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(3RS,5aR,6R)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]acetamide.<sup>k</sup> (6R,7R)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>l</sup> (6R,7R)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>m</sup> (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>n</sup> (6R,7R)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>o</sup> Cefdinir decarboxy open ring lactone is a mixture of two isomers labeled cefdinir decarboxy open ring lactone a and b. The sum of the values is reported. The limit for the sum of the two isomers is 1.0%.<sup>p</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(2RS,5RS)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]methyl]acetamide.

Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefdinir decarboxy open ring lactone a <sup>o,p</sup>	1.62	1.0	1.0
Cefdinir decarboxy open ring lactone b <sup>o,p</sup>	1.64	1.0	
Cefdinir impurity 3 <sup>l</sup>	1.82	1.0	0.2
Individual unidentified impurities	—	1.0	0.2
Total impurities	—	—	5.0

<sup>a</sup> N-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.<sup>b</sup> (Z)-2-(2-Aminothiazol-4-yl)-N-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.<sup>c</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>d</sup> (R,Z)-2-[(R)-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido](carboxymethyl)-5-ethylidene-5,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.<sup>e</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>f</sup> Cefdinir impurity 1, cefdinir impurity 2, and cefdinir impurity 3 are unidentified impurities.<sup>g</sup> Cefdinir related compound A is a mixture of four isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported. The limit for the sum of the four isomers is 2.5%.<sup>h</sup> 2(R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2RS,5RS)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid.<sup>i</sup> (6R,7S)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>j</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(3RS,5aR,6R)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]acetamide.<sup>k</sup> (6R,7R)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>l</sup> (6R,7R)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>m</sup> (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>n</sup> (6R,7R)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>o</sup> Cefdinir decarboxy open ring lactone is a mixture of two isomers labeled cefdinir decarboxy open ring lactone a and b. The sum of the values is reported. The limit for the sum of the two isomers is 1.0%.<sup>p</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(2RS,5RS)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]methyl]acetamide.**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Cefdinir RS

USP Cefdinir Related Compound A RS

(2R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2RS,5RS)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid (three other stereoisomers are also present in this RS).

C<sub>14</sub>H<sub>15</sub>N<sub>5</sub>O<sub>6</sub>S<sub>2</sub> 413.43

USP Cefdinir Related Compound B RS

(6R,7R)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

C<sub>14</sub>H<sub>13</sub>N<sub>4</sub>O<sub>4</sub>S<sub>2</sub> 365.41**Cefdinir for Oral Suspension****DEFINITION**

Cefdinir for Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of cefdinir (C<sub>14</sub>H<sub>13</sub>N<sub>5</sub>O<sub>6</sub>S<sub>2</sub>). It may contain one or more suitable buffers, flavors, pre-



servatives, stabilizing agents, sweeteners, and suspending agents.

## IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

## ASSAY

### • PROCEDURE

**Buffer:** 10.7 mg/mL of anhydrous dibasic sodium phosphate and 3.4 mg/mL of monobasic potassium phosphate in water. Adjust with phosphoric acid or sodium hydroxide to a pH of  $7.0 \pm 0.05$  before final dilution.

**Solution A:** 7 mg/mL of citric acid monohydrate. Adjust with phosphoric acid to a pH of  $2.0 \pm 0.05$ .

**Mobile phase:** Methanol, tetrahydrofuran, and *Solution A* (111:28:1000)

**System suitability solution:** 50 µg/mL of USP Cefdinir RS and 175 µg/mL of *m*-hydroxybenzoic acid in *Buffer*

**Standard solution:** 50 µg/mL of USP Cefdinir RS in *Buffer*

**Sample solution:** Equivalent to 50 µg/mL of cefdinir from constituted Cefdinir for Oral Suspension in *Buffer*

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 15-cm; 4-µm packing L1

**Flow rate:** 1.4 mL/min

**Injection volume:** 15 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 3.0 between cefdinir and *m*-hydroxybenzoic acid, *System suitability solution*

**Tailing factor:** NMT 2.0 for cefdinir, *System suitability solution*

**Relative standard deviation:** NMT 1.0% for cefdinir, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ) in the portion of Cefdinir for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cefdinir from the *Sample solution*

$r_S$  = peak response of cefdinir from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of cefdinir in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

**Medium:** 0.05 M phosphate buffer, pH 6.8; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Detector:** UV 290 nm

**Standard solution:** 0.14 mg/mL of USP Cefdinir RS in *Medium*

**Sample solution:** Transfer 5 mL, by weight, of the reconstituted Cefdinir for Oral Suspension into the vessel. After the appropriate time, withdraw a portion of the solution under test, and pass through a suitable filter of 0.45-µm pore size. Dilute a portion of each filtered sample with *Medium* as necessary to obtain a solution having a concentration of about 0.14 mg/mL of cefdinir.

**Blank:** *Medium*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times (d/W_U) \times V \times D \times (1/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$d$  = density of Cefdinir for Oral Suspension (mg/mL)

$W_U$  = weight of reconstituted Cefdinir for Oral Suspension taken (mg)

$V$  = volume of *Medium*, 900 mL

$D$  = dilution factor of the *Sample solution* (mL/mL)

$L$  = label claim (mg/mL)

**Tolerances:** NLT 80% (Q) of the labeled amount of cefdinir ( $C_{14}H_{13}N_5O_5S_2$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905)

For single-unit containers

Acceptance criteria: Meets the requirements

### • DELIVERABLE VOLUME (698)

For multiple-unit containers

Acceptance criteria: Meets the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

**Solution A:** 14.2 mg/mL of anhydrous dibasic sodium phosphate

**Solution B:** 13.6 mg/mL of monobasic potassium phosphate

**Buffer:** Combine appropriate amounts of *Solution A* and *Solution B* (about 2:1) to obtain a solution with a pH of  $7.0 \pm 0.1$ .

**Solution C:** Dilute tetramethylammonium hydroxide (10% aqueous) with water to obtain a 0.1% solution. Adjust with dilute phosphoric acid (1 in 10) to a pH of  $5.5 \pm 0.1$ .

**Solution D:** 37.2 mg/mL of edetate disodium

**Solution E:** To 1000 mL of *Solution C* add 0.4 mL of *Solution D*.

**Solution F:** Acetonitrile, methanol, *Solution C*, and *Solution D* (150: 100: 250: 0.2)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution E (%)	Solution F (%)
0	95	5
2	95	5
22	75	25
32	50	50
37	50	50
38	95	5
58	95	5

**System suitability stock solution 1:** 40 µg/mL of USP Cefdinir Related Compound A RS in *Solution C*

**System suitability stock solution 2:** 40 µg/mL of USP Cefdinir Related Compound B RS in *Buffer*

**System suitability solution:** Transfer 37.5 mg of USP Cefdinir RS to a 25-mL volumetric flask, and add about 10 mL of *Buffer*. Add 5.0 mL each of *System suitability stock solution 1* and *System suitability stock solution 2*, and dilute with *Solution C* to volume.

**Standard stock solution:** 750 µg/mL of USP Cefdinir RS in *Buffer*

**Standard solution:** 15 µg/mL of USP Cefdinir RS from the *Standard stock solution* in *Solution C*



**Sample solution:** Transfer a quantity equivalent to 150 mg of cefdinir from the constituted Cefdinir for Oral Suspension to a 100-mL volumetric flask. Dissolve in 30 mL of *Buffer*, and dilute with *Solution C* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Temperatures**

**Autosampler:** 4°

**Column:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between cefdinir and the third peak of USP Cefdinir Related Compound A RS, *System suitability solution*

**Tailing factor:** NMT 1.5 for cefdinir related compound B, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the cefdinir peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cefdinir for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (100/F)$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of cefdinir from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefdinir in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*. The reporting threshold is 0.1%.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Thiazolylacetyl glycine oxime <sup>a</sup>	0.10	1.0	0.5
Thiazolylacetyl glycine oxime acetal <sup>b</sup>	0.13	1.0	0.6
Cefdinir sulfoxide <sup>c</sup>	0.36	1.0	0.2
Cefdinir thiazine analog <sup>d</sup>	0.46	0.68	0.3
3-Methyl cefdinir <sup>e</sup>	0.75	1.0	0.7
Cefdinir impurity 1 <sup>f</sup>	0.77	1.0	0.2
Cefdinir related compound A (cefdinir open ring lactone a) <sup>g,h</sup>	0.85	0.65	3.3
Cefdinir related compound A (cefdinir open ring lactone b) <sup>g,h</sup>	0.94	0.65	
Cefdinir related compound A (cefdinir open ring lactone c) <sup>g,h</sup>	1.11	0.65	
Cefdinir related compound A (cefdinir open ring lactone d) <sup>g,h</sup>	1.14	0.65	
7S-Cefdinir <sup>i</sup>	1.18	1.0	0.2
Cefdinir lactone <sup>j</sup>	1.23	1.0	0.8
Cefdinir related compound B <sup>k</sup>	1.28	1.0	0.2
Cefdinir isoxazole analog <sup>l</sup>	1.37	0.72	0.5
Cefdinir impurity 2 <sup>l</sup>	1.44	1.0	0.2
Cefdinir glyoxalic analog <sup>m</sup>	1.49	1.0	0.2

<sup>a</sup> N-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.

<sup>b</sup> (Z)-2-(2-Aminothiazol-4-yl)-N-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.

<sup>c</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>d</sup> (R,Z)-2-[(R)-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido](carboxy)methyl]-5-ethylidene-5,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.

<sup>e</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> Cefdinir impurity 1, cefdinir impurity 2, and cefdinir impurity 3 are unidentified impurities.

<sup>g</sup> Cefdinir related compound A is a mixture of four isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported; the limit for the sum of the four isomers is 3.3%.

<sup>h</sup> 2(R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2R,5R)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid.

<sup>i</sup> (6R,7S)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>j</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(3R,5aR,6R)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]acetamide.

<sup>k</sup> (6R,7R)-7-[(Z)-2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>l</sup> (6R,7R)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>m</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>n</sup> (6R,7R)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>o</sup> Cefdinir decarboxy open ring lactone is a mixture of two isomers labeled cefdinir decarboxy open ring lactone a and b. The sum of the values is reported; the limit for the sum of the two isomers is 1.1%.

<sup>p</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(2R,5R)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]methyl]acetamide.



Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
E-Cefdinir <sup>a</sup>	1.51	1.0	1.4
Cefdinir decarboxy open ring lactone <sup>a,p</sup>	1.62	1.0	1.1
Cefdinir decarboxy open ring lactone <sup>b,p</sup>	1.64	1.0	
Cefdinir impurity 3 <sup>i</sup>	1.82	1.0	0.2
Individual unidentified impurities	—	1.0	0.2
Total impurities	—	—	6.2

<sup>a</sup> N-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]glycine.<sup>b</sup> (Z)-2-(2-Aminothiazol-4-yl)-N-(2,2-dihydroxyethyl)-2-(hydroxyimino)acetamide.<sup>c</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-5,8-dioxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>d</sup> (R,Z)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido](carboxymethyl)-5-ethylidene-5,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.<sup>e</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>f</sup> Cefdinir impurity 1, cefdinir impurity 2, and cefdinir impurity 3 are unidentified impurities.<sup>g</sup> Cefdinir related compound A is a mixture of four isomers labeled cefdinir open ring lactones a, b, c, and d. The sum of the values is reported; the limit for the sum of the four isomers is 3.3%.<sup>h</sup> 2(R,Z)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2RS,5RS)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid.<sup>i</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>j</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(3RS,5aR,6R)-3-methyl-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]acetamide.<sup>k</sup> (6R,7R)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>l</sup> (6R,7R)-7-(4-Hydroxyisoxazole-3-carboxamido)-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>m</sup> (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-oxoacetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>n</sup> (6R,7R)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.<sup>o</sup> Cefdinir decarboxy open ring lactone is a mixture of two isomers labeled cefdinir decarboxy open ring lactone a and b. The sum of the values is reported; the limit for the sum of the two isomers is 1.1%.<sup>p</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)-N-[(2RS,5RS)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]methyl]acetamide.**SPECIFIC TESTS**

- **pH (791):** 3.2–4.8

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**

USP Cefdinir RS

USP Cefdinir Related Compound A RS

(2R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-2-[(2RS,5RS)-5-methyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl]acetic acid (three other stereoisomers are also present in this RS).

C<sub>14</sub>H<sub>15</sub>N<sub>5</sub>O<sub>6</sub>S<sub>2</sub> 413.43

USP Cefdinir Related Compound B RS

(6R,7R)-7-[2-(2-Amino-4-thiazolyl)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

C<sub>14</sub>H<sub>13</sub>N<sub>4</sub>O<sub>4</sub>S<sub>2</sub> 365.41**Cefepime for Injection****DEFINITION**

Cefepime for Injection is a sterile mixture of Cefepime Hydrochloride and Arginine. It contains the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of cefepime (C<sub>19</sub>H<sub>24</sub>N<sub>6</sub>O<sub>5</sub>S<sub>2</sub>).

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 20 mg/mL of arginine

**Sample solution:** 40 mg/mL of Cefepime for Injection

**Developing solvent system:** *n*-Propyl alcohol, ammonium hydroxide, and water (7:4:5)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed in *Thin-Layer Chromatographic Identification Test (201)*, except to spray the plate with ninhydrin TS.

**Acceptance criteria:** Arginine appears as a dark red spot. The intensity and the R<sub>f</sub> value of the spot from the *Sample solution* correspond to those from the *Standard solution*.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- **PROCEDURE**

**Solution A:** 0.68 mg/mL of monobasic potassium phosphate in water

**Solution B:** Acetonitrile and *Solution A* (1:9), adjusted with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0

**Solution C:** Acetonitrile and *Solution A* (1:1), adjusted with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0

**Mobile phase:** See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
10	100	0
30	50	50
35	50	50
36	100	0
45	100	0

**Standard solution:** 1.4 mg/mL of USP Cefepime Hydrochloride RS in *Solution B*. [NOTE—Sonicate if necessary. Inject immediately or store in a refrigerator and use within 12 h.]

**Sample solution:** Constitute one container of Cefepime for Injection as directed on the label, and dilute using *Solution B* to 1 mg/mL of cefepime. [NOTE—For products that are designed for administration with a syringe, withdraw the entire withdrawable contents of the vial and transfer to a suitable volumetric flask. Dilute with *Solution B* to volume. For all other types, transfer the contents of the reconstituted vial quantitatively to a suitable volumetric flask, and dilute with *Solution B* to volume.]



**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Flow rate:** 1 mL/min**Injection size:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of C<sub>19</sub>H<sub>24</sub>N<sub>6</sub>O<sub>5</sub>S<sub>2</sub> in the portion of Cefepime for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Cefepime Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of cefepime in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–115.0%**PERFORMANCE TESTS**

- UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

**IMPURITIES****Organic Impurities**

- PROCEDURE 1: LIMIT OF N-METHYLPYRROLIDINE**

**Mobile phase:** Acetonitrile and 0.01 N nitric acid (1:19)**Standard solution:** 0.05 mg/mL of *N*-methylpyrrolidine in 0.002 N nitric acid**Sample solution:** Equivalent to 5 mg/mL of cefepime hydrochloride in 0.002 N nitric acid. [NOTE—Inject this solution immediately.]**Chromatographic system**(See *Chromatography* <621>, *System Suitability*.)**Mode:** LC**Detector:** Conductivity**Column:** 4.0-mm × 25-cm; 5-μm packing L76**Flow rate:** 1 mL/min**Injection size:** 10 μL**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 4.0%**Analysis****Samples:** *Standard solution* and *Sample solution*[NOTE—Record the chromatogram of the *Sample solution* for about 6 times the retention time of the *N*-methylpyrrolidine peak.]Calculate the percentage of *N*-methylpyrrolidine in the portion of Cefepime for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of *N*-methylpyrrolidine from the *Sample solution* $r_S$  = peak response of *N*-methylpyrrolidine from the *Standard solution* $C_S$  = concentration of *N*-methylpyrrolidine in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of cefepime in the *Sample solution* (mg/mL)**Acceptance criteria:** NMT 1.0%

- PROCEDURE 2: OTHER ORGANIC IMPURITIES**

**Solution A, Solution B, Solution C, Mobile phase, Chromatographic system, and Sample solution:** Prepare as directed for Assay.**System suitability solution:** 1.4 mg/mL of USPCefepime Hydrochloride RS and 15 μg/mL each of USP Cefepime Related Compound D RS and USP Cefepime Related Compound E RS in *Solution B***System suitability****Sample:** *System suitability solution***Suitability requirements**[NOTE—See *Impurity Table 1* for the relative retention times.]**Resolution:** NLT 2.0 between cefepime related compound E and cefepime related compound D**Tailing factor:** NMT 1.5, cefepime**Analysis****Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Cefepime for Injection taken:

$$\text{Result} = (r_U/r_T) \times 1/F \times 100$$

 $r_U$  = peak response for each impurity $r_T$  = sum of the peak responses from the chromatogram $F$  = relative response factor from *Impurity Table 1***Acceptance criteria**

[NOTE—The reporting level is 0.2% for cefepime impurity C and 0.05% for all other related compounds.]

**Individual impurities:** See *Impurity Table 1*.**Total impurities:** NMT 2.2%. [NOTE—Total impurities include *N*-methylpyrrolidine.]**Impurity Table 1**

Name	Relative Retention Time (%)	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefepime amine derivative <sup>a,b</sup> (cefepime related compound E)	0.4	—	—
Thiazolylglyoxalic methyloxime <sup>a,c</sup> (cefepime related compound D)	0.5	—	—
Thiazolylloxime acetaldehyde <sup>d</sup> (cefepime related compound C)	0.6	0.63	0.5
Cefepime dimer <sup>a,e</sup> (cefepime related compound F)	0.8	—	—
Cefepime	1.0	—	—
<i>E</i> -Cefepime <sup>f</sup> (cefepime related compound A)	2.7	0.71	0.5

<sup>a</sup> These impurities are synthetic process impurities that are controlled in the drug substance. They are listed here for reference only.<sup>b</sup> (6*R*,7*R*)-7-Amino-3-[(1-methylpyrrolidin-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.<sup>c</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.<sup>d</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)-*N*-(2-oxoethyl)acetamide.<sup>e</sup> 1-[[[(6*R*,7*R*)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-[(6*R*,7*R*)-2-carboxy-3-[(1-methylpyrrolidin-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylcarbamoyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.<sup>f</sup> 1-[[[(6*R*,7*R*)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.<sup>g</sup> 1-[[[(6*R*,7*R*)-7-[(Z)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]thiazol-4-yl]-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.



Impurity Table 1 (Continued)

Name	Relative Retention Time (%)	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefepime dioxime <sup>a</sup> (cefepime related compound B)	4.3	—	—
Any individual unspecified impurity	—	1.0	0.5

<sup>a</sup> These impurities are synthetic process impurities that are controlled in the drug substance. They are listed here for reference only.

<sup>b</sup> (6*R*,7*R*)-7-Amino-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

<sup>c</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.

<sup>d</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)-N-(2-oxoethyl)acetamide.

<sup>e</sup> 1-[[[(6*R*,7*R*)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-[(6*R*,7*R*)-2-carboxy-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylcarbamoyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.

<sup>f</sup> 1-[[[(6*R*,7*R*)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.

<sup>g</sup> 1-[[[(6*R*,7*R*)-7-[(Z)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]thiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.

## SPECIFIC TESTS

- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** (1), *Specific Tests*, *Completeness and clarity of solutions*: At the time of use, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.06 USP Endotoxin Unit/mg of cefepime
- **STERILITY TESTS** (71): Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*
- **pH** (791): 4.0–6.0, in a solution containing 100 mg/mL of cefepime
- **WATER DETERMINATION**, *Method I* (921): NMT 4.0%
- **OTHER REQUIREMENTS**: Meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*

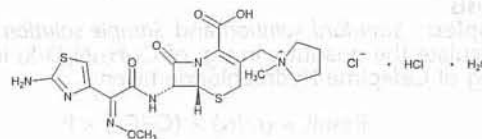
## ADDITIONAL REQUIREMENTS

### Change to read:

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers as described under *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for constitution* (CN 1-May-2017), and store in a refrigerator or at controlled room temperature. Store reconstituted solution in a refrigerator for NMT 7 days.
- **LABELING**: Label it to indicate that it is to be diluted with a suitable parenteral vehicle before intravenous infusion.
- **USP REFERENCE STANDARDS** (11)  
USP Cefepime Hydrochloride RS  
USP Cefepime Related Compound D RS  
Thiazolylglyoxalic methyloxime; (Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetic acid.  
C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O<sub>3</sub>S 201.20

USP Cefepime Related Compound E RS  
Cefepime amine derivative; 1-[[[(6*R*,7*R*)-7-amino-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium-1-ium chloride.  
C<sub>13</sub>H<sub>20</sub>ClN<sub>3</sub>O<sub>3</sub>S 333.83  
USP Endotoxin RS

## Cefepime Hydrochloride



C<sub>19</sub>H<sub>25</sub>ClN<sub>6</sub>O<sub>5</sub>S<sub>2</sub> · HCl · H<sub>2</sub>O 571.50  
Pyrrolidinium, 1-[[7-[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methyl-, chloride, monohydrochloride, monohydrate, [6*R*-[6*α*,7*β*(Z)]]-;  
1-[[[(6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride, 7*Z*-(Z)-(O-methyloxime), monohydrochloride, monohydrate [123171-59-5].

## DEFINITION

Cefepime Hydrochloride contains the equivalent of NLT 825 µg and NMT 911 µg of cefepime (C<sub>19</sub>H<sub>24</sub>N<sub>6</sub>O<sub>5</sub>S<sub>2</sub>)/mg, calculated on the anhydrous basis.

## IDENTIFICATION

- **INFRARED ABSORPTION** (197M)  
Sample: Proceed as directed in the chapter, but do not dry.

## ASSAY

- **PROCEDURE**  
**Solution A:** 0.68 mg/mL of monobasic potassium phosphate in water  
**Solution B:** Acetonitrile and *Solution A* (1:9). Adjust with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0.  
**Solution C:** Acetonitrile and *Solution A* (1:1). Adjust with 2% phosphoric acid or 2% potassium hydroxide to a pH of 5.0.  
**Mobile phase:** See the gradient table below.

Time (min)	Solution B (%)	Solution C (%)
0	100	0
10	100	0
30	50	50
35	50	50
36	100	0
45	100	0

**Standard solution:** 1.4 mg/mL of USP Cefepime Hydrochloride RS in *Solution B*. [NOTE—Sonicate if necessary. Inject immediately or store in a refrigerator and use within 12 h.]

**Sample solution:** 1.4 mg/mL of Cefepime Hydrochloride in *Solution B*. [NOTE—Sonicate if necessary. Inject immediately or store in a refrigerator and use within 12 h.]



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection size: 10 μL

**System suitability**Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the quantity, in μg, of C<sub>19</sub>H<sub>24</sub>N<sub>6</sub>O<sub>5</sub>S<sub>2</sub> in each mg of Cefepime Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Cefepime Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Cefepime Hydrochloride in the *Sample solution* (mg/mL) $P$  = content of cefepime in USP Cefepime Hydrochloride RS (μg/mg)

Acceptance criteria: 825–911 μg/mg on the anhydrous basis

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION** (281): NMT 0.1%

**Delete the following:**

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm (Official 1, Jan-2018)

**Organic Impurities**

- **PROCEDURE 1: LIMIT OF N-METHYLPYRROLIDINE**

Mobile phase: Acetonitrile and 0.01 N nitric acid (1:19)

Standard solution: 0.05 mg/mL of *N*-methylpyrrolidine in 0.002 N nitric acid

Sample solution: Equivalent to 5 mg/mL of cefepime hydrochloride in 0.002 N nitric acid. [NOTE—Inject this solution immediately.]

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: Conductivity

Column: 4.0-mm × 25-cm; 5-μm packing L76

Flow rate: 1 mL/min

Injection size: 10 μL

**System suitability**Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 4.0%

**Analysis**Samples: *Standard solution* and *Sample solution*[NOTE—Record the chromatogram of the *Sample solution* for about 6 times the retention time of the *N*-methylpyrrolidine peak.]Calculate the percentage of *N*-methylpyrrolidine in the portion of Cefepime Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of *N*-methylpyrrolidine from the *Sample solution* $r_S$  = peak response of *N*-methylpyrrolidine from the *Standard solution* $C_S$  = concentration of *N*-methylpyrrolidine in the *Standard solution* (mg/mL) $C_U$  = concentration of Cefepime Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.3%

- **PROCEDURE 2: OTHER ORGANIC IMPURITIES**

Solution A, Solution B, Solution C, Mobile phase,

Sample solution and Chromatographic system: Prepare as directed in the Assay.

System suitability solution: 1.4 mg/mL of USP

Cefepime Hydrochloride RS and 15 μg/mL each of USP

Cefepime Related Compound D RS and USP Cefepime

Related Compound E RS in *Solution B***System suitability**Samples: *System suitability solution*

Suitability requirements

[NOTE—See *Impurity Table 1* for the relative retention times.]

Resolution: NLT 2.0 between cefepime related compound E and cefepime related compound D

Tailing factor: NMT 1.5, cefepime

**Analysis**Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Cefepime Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 1/F \times 100$$

 $r_U$  = peak response of each impurity $r_T$  = sum of the peak responses for all the peaks in the chromatogram $F$  = relative response factor from *Impurity Table 1***Acceptance criteria**

[NOTE—The reporting level is 0.2% for cefepime impurity C and 0.05% for all other related compounds.]

Individual impurities: See *Impurity Table 1*.Total impurities: NMT 1.0%. [NOTE—Total impurities does not include *N*-methylpyrrolidine.]**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefepime amine derivative <sup>a</sup> (cefepime related compound E)	0.4	0.48	0.1
Thiazolylglyoxalic methyloxime <sup>b</sup> (cefepime related compound D)	0.5	1.0	0.1
Thiazolylloxime acetaldehyde <sup>c</sup> (cefepime related compound C)	0.6	0.63	0.3
Cefepime dimer <sup>d</sup> (cefepime related compound F)	0.8	1.0	0.2
Cefepime	1.0	—	—
E-Cefepime <sup>e</sup> (cefepime related compound A)	2.7	0.71	0.3

<sup>a</sup> (6*R*,7*R*)-7-Amino-3-[(1-methylpyrrolidin-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.<sup>b</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.<sup>c</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)-*N*-(2-oxoethyl)acetamide.<sup>d</sup> 1-[(6*R*,7*R*)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-[(6*R*,7*R*)-2-carboxy-3-[(1-methylpyrrolidin-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylcarbonyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.<sup>e</sup> 1-[(6*R*,7*R*)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.<sup>f</sup> 1-[(6*R*,7*R*)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]thiazol-4-yl]-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-methylpyrrolidinium chloride.



Impurity Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cefepime dioxime <sup>a</sup> (cefepime related compound B)	4.3	0.71	0.2
Any individual unspecified impurity	—	1.0	0.1

<sup>a</sup> (6*R*,7*R*)-7-Amino-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

<sup>b</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.

<sup>c</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)-*N*-(2-oxoethyl)acetamide.

<sup>d</sup> 1-[(6*R*,7*R*)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-[(6*R*,7*R*)-2-carboxy-3-[(1-methylpyrrolidinium-1-yl)methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylcarbonyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl]-1-methylpyrrolidinium chloride.

<sup>e</sup> 1-[(6*R*,7*R*)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl]-1-methylpyrrolidinium chloride.

<sup>f</sup> 1-[(6*R*,7*R*)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]thiazol-4-yl)-2-(methoxyimino)acetamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl]-1-methylpyrrolidinium chloride.

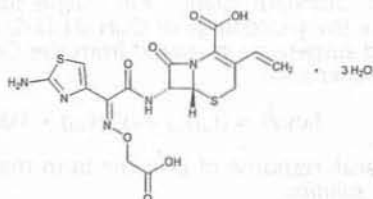
### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TESTS (85):** Where the label states that Cefepime Hydrochloride is sterile or that it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.04 USP Endotoxin Unit/mg of cefepime hydrochloride.
- **STERILITY TESTS (71):** Where the label states that Cefepime Hydrochloride is sterile, it meets the requirements when tested as directed in the *Test for Sterility of the Product to be Examined, Membrane Filtration*.
- **CRYSTALLINITY (695):** Meets the requirements
- **WATER DETERMINATION, Method I (921):** 3.0%–4.5%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS (11)**
  - USP Cefepime Hydrochloride RS
  - USP Cefepime Related Compound D RS
  - Thiazolylglyoxalic methyloxime; (Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetic acid.
  - C<sub>6</sub>H<sub>7</sub>N<sub>3</sub>O<sub>3</sub>S 201.20
  - USP Cefepime Related Compound E RS
  - Cefepime amine derivative; 1-[(6*R*,7*R*)-7-amino-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl]-1-methylpyrrolidin-1-ium chloride.
  - C<sub>13</sub>H<sub>20</sub>ClN<sub>3</sub>O<sub>3</sub>S 333.83
  - USP Endotoxin RS

## Cefixime

C<sub>16</sub>H<sub>15</sub>N<sub>5</sub>O<sub>7</sub>S<sub>2</sub> · 3H<sub>2</sub>O

507.50

C<sub>16</sub>H<sub>15</sub>N<sub>5</sub>O<sub>7</sub>S<sub>2</sub> (anhydrous) 453.46  
 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(2-amino-4-thiazolyl)[(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-, trihydrate, [6*R*:[6*α*,7*β*(Z)]]-; (6*R*,7*R*)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7*z*-(Z)-[O-(carboxymethyl)oxime]trihydrate [125110-14-7].  
 Anhydrous [79350-37-1].

### DEFINITION

Cefixime contains the equivalent of NLT 950 μg/mg and NMT 1030 μg/mg of cefixime (C<sub>16</sub>H<sub>15</sub>N<sub>5</sub>O<sub>7</sub>S<sub>2</sub>), calculated on the anhydrous basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Sample:** Dissolve 5 mg by trituration in 2 mL of methanol, and evaporate with the aid of gentle heat to dryness.

**Acceptance criteria:** Meets the requirements

### ASSAY

#### • PROCEDURE

**Solution A:** 25 mL of 0.4 M tetrabutylammonium hydroxide solution diluted with water to 1000 mL, and adjusted with 1.5 M phosphoric acid to a pH of 6.5

**Solution B:** 13.6 g/L of monobasic potassium phosphate in water

**Solution C:** 14.2 g/L of anhydrous dibasic sodium phosphate in water

**Buffer:** Adjust an aliquot of *Solution C* with *Solution B* to a pH of 7.0.

**Mobile phase:** Acetonitrile and *Solution A* (1:3)

**System suitability solution:** 1 mg/mL of USP Cefixime RS in water. Heat this solution at 95° in an oil bath for 45 min, cool, and use promptly.

**Standard solution:** 0.2 mg/mL of cefixime from USP Cefixime RS in *Buffer*. Use this solution promptly.

**Sample solution:** 0.22 mg/mL of Cefixime in *Buffer*. Use this solution promptly.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 12.5-cm; 4-μm packing L1

**Column temperature:** 40°

**Flow rate:** Adjusted so that the retention time of cefixime is 10 min

**Injection volume:** 10 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cefixime (*E*)-isomer and cefixime are about 0.9 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between cefixime and cefixime (*E*)-isomer, *System suitability solution*

**Column efficiency:** NLT 4000 theoretical plates, *Standard solution*

Calculate as follows:

$$\text{Result} = 5.545(t/W_{H/2})^2$$

*t* = retention time

*W*<sub>H/2</sub> = peak width at half height

**Tailing factor:** NLT 0.9 and NMT 2.0 for the analyte peak, *Standard solution*

Calculate as follows:

$$\text{Result} = W_{0.1}/2f$$

*W*<sub>0.1</sub> = width of peak of 10% height

*f* = distance from the peak maximum to the leading edge of the peak measured at 10% of the peak height



Relative standard deviation: NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the quantity, in  $\mu\text{g}/\text{mg}$ , of cefixime ( $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_7\text{S}_2$ ) in the portion of Cefixime taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Cefixime RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Cefixime in the *Sample solution* (mg/mL)  
 $P$  = potency of cefixime in USP Cefixime RS (mg/mg)  
 $F$  = conversion factor, 1000  $\mu\text{g}/\text{mg}$

**Acceptance criteria:** 950–1030  $\mu\text{g}/\text{mg}$  on the anhydrous basis

#### IMPURITIES

##### • ORGANIC IMPURITIES

*Solution A, Solution B, Solution C, Buffer, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:* Proceed as directed in the Assay.

#### Analysis

**Samples:** *Sample solution*

Calculate the percentage of each impurity in the portion of Cefixime taken:

$$\text{Result} = (r_U/r_S) \times P \times F \times 100$$

- $r_U$  = peak area for each impurity  
 $r_S$  = cefixime peak area  
 $P$  = potency of cefixime calculated in the Assay ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

#### Acceptance criteria

**Individual impurities:** NMT 1.0% of any individual impurity is found.

**Total impurities:** NMT 2.0%

#### SPECIFIC TESTS

##### • OPTICAL ROTATION, *Specific Rotation* (781S)

**Diluent:** 20-mg/mL solution of sodium bicarbonate

**Sample solution:** 10 mg/mL in *Diluent*

**Acceptance criteria:**  $-75^\circ$  to  $-88^\circ$

##### • CRYSTALLINITY (695): Meets the requirements

##### • PH (791)

**Sample solution:** 0.7 mg/mL of cefixime

**Acceptance criteria:** 2.6–4.1

##### • WATER DETERMINATION, *Method I* (921): 9.0%–12.0%

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE: Preserve in tight containers.

**LABELING:** Label to indicate that it is the trihydrate form. Where the quantity of Cefixime is indicated in the labeling of any preparation containing Cefixime, this shall be understood to be in terms of anhydrous cefixime ( $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_7\text{S}_2$ ).

##### • USP REFERENCE STANDARDS (11)

USP Cefixime RS

ime ( $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_7\text{S}_2$ )/mL when constituted as directed in the labeling.

#### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Solution A:** 0.4 M tetrabutylammonium hydroxide solution and water (1:39). Adjust with 1.5 M phosphoric acid to a pH of 6.5.

**Solution B:** 13.6 mg/mL of monobasic potassium phosphate

**Solution C:** 14.2 mg/mL of anhydrous dibasic sodium phosphate. Adjust a volume of this solution with a sufficient volume of *Solution B* to a pH of 7.0.

**Mobile phase:** Acetonitrile and *Solution A* (1:3)

**System suitability solution:** 1 mg/mL of USP Cefixime RS. [NOTE—Heat this solution at  $95^\circ$  in an oil bath for 45 min, cool, and use promptly.]

**Standard solution:** 0.2 mg/mL of USP Cefixime RS in *Solution C*. [NOTE—Use this solution promptly.]

**Sample solution:** Constitute Cefixime for Oral Suspension as directed in the labeling. Quantitatively dilute a suitable aliquot of the suspension, freshly mixed and free from air bubbles, with *Solution C* to obtain a solution having a nominal concentration of 0.2 mg of cefixime/mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  12.5-cm; 4- $\mu\text{m}$  packing L1

**Temperature:**  $40^\circ$

**Flow rate:** Adjust flow rate so that the retention time of cefixime is about 10 min.

**Injection size:** 10  $\mu\text{L}$

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cefixime (E)-isomer and cefixime are about 0.9 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between cefixime and cefixime (E)-isomer, *System suitability solution*

**Column efficiency:** NLT 4000 theoretical plates, *Standard solution*. Use the following formula to calculate column efficiency:

$$\text{Result} = 5.545(t/W_{0.1})^2$$

**Tailing factor:** NLT 0.9 and NMT 2.0 for the analyte peak, *Standard solution*. Use the following formula to calculate tailing factor:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$  = peak width at 10% peak height

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $\text{C}_{16}\text{H}_{15}\text{N}_5\text{O}_7\text{S}_2$  in the constituted suspension prepared from the Cefixime for Oral Suspension:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of cefixime from the *Sample solution*  
 $r_S$  = peak response of cefixime from the *Standard solution*  
 $C_S$  = concentration of USP Cefixime RS in the *Standard solution* (mg/mL)

## Cefixime for Oral Suspension

#### DEFINITION

Cefixime for Oral Suspension is a dry mixture of Cefixime and one or more suitable diluents, flavors, preservatives, and suspending agents. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cefix-



$C_U$  = nominal concentration of cefixime in the  
Sample solution (mg/mL)

Acceptance criteria: 90.0%–120.0%

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905)** FOR SOLIDS PACKAGED IN SINGLE-UNIT CONTAINERS: Meets the requirements
- **DELIVERABLE VOLUME (698)**: Meets the requirements

### SPECIFIC TESTS

- **PH (791)**: 2.5–4.5, in the suspension constituted as directed in the labeling

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **LABELING**: Label it to indicate that the cefixime contained therein is in the trihydrate form.
- **USP REFERENCE STANDARDS (11)**  
USP Cefixime RS

## Cefixime Tablets

### DEFINITION

Cefixime Tablets contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of cefixime ( $C_{16}H_{15}N_5O_7S_2$ ).

### IDENTIFICATION

- **A**. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Solution A**: 25 mL of 0.4 M tetrabutylammonium hydroxide solution diluted with water to 1000 mL, and adjusted with 1.5 M phosphoric acid to a pH of 6.5

**Solution B**: 13.6 g/L of monobasic potassium phosphate in water

**Solution C**: 14.2 g/L of anhydrous dibasic sodium phosphate in water

**Buffer**: Adjust an aliquot of *Solution C* with *Solution B* to a pH of 7.0.

**Mobile phase**: Acetonitrile and *Solution A* (1:3)

**System suitability solution**: 1 mg/mL of USP Cefixime RS in water. Heat this solution at 95° in an oil bath for 45 min, cool, and use promptly.

**Standard solution**: 0.2 mg/mL of USP Cefixime RS in *Buffer*. Use this solution promptly.

**Sample stock solution**: Nominally 4 mg/mL of cefixime in *Buffer* from finely powdered Tablets (NLT 20). Sonicate as required, and centrifuge.

**Sample solution**: Nominally 0.2 mg/mL of cefixime from *Sample stock solution* in *Buffer*

#### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode**: LC

**Detector**: UV 254 nm

**Column**: 4.6-mm × 12.5-cm; 4-μm packing L1

**Column temperature**: 40°

**Flow rate**: Adjusted so that the retention time of cefixime is about 10 min

**Injection volume**: 10 μL

#### System suitability

**Samples**: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cefixime (E)-isomer and cefixime are about 0.9 and 1.0, respectively.]

### Suitability requirements

**Resolution**: NLT 2.0 between cefixime and cefixime (E)-isomer, *System suitability solution*

**Column efficiency**: NLT 4000 theoretical plates for the *Standard solution*

Calculate as follows:

$$\text{Result} = 5.545(t/W_{h/2})^2$$

$t$  = retention time

$W_{h/2}$  = peak width at half height

**Tailing factor**: NLT 0.9 and NMT 2.0 for the analyte peak

Calculate as follows:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$  = width of peak at 10% height

$f$  = distance from the peak maximum to the leading edge of the peak measured at 10% of the peak height

**Relative standard deviation**: NMT 2.0%, *Standard solution*

### Analysis

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefixime ( $C_{16}H_{15}N_5O_7S_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cefixime RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefixime in the *Sample solution* (mg/mL)

$P$  = potency of cefixime in USP Cefixime RS (mg/mg)

Acceptance criteria: 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Medium**: 6.8 g/L of monobasic potassium phosphate in water, adjusted with 1 N sodium hydroxide to a pH of 7.2; 900 mL

**Apparatus 1**: 100 rpm

**Time**: 45 min

**Detector**: UV 288 nm

**Standard solution**: USP Cefixime RS in *Medium*. An amount of methanol not to exceed 0.1% of the total volume of the *Standard solution* may be used to bring the USP Cefixime RS into solution before dilution with *Medium*, and the solution may be sonicated to ensure complete dissolution of the USP Cefixime RS.

**Sample solution**: Sample per *Dissolution (711)*. Dilute with *Medium* to a concentration similar to that of the *Standard solution*.

**Tolerances**: NLT 75% (Q)

- **UNIFORMITY OF DOSAGE UNITS (905)**: Meet the requirements

### SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921)**: NMT 10.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **LABELING**: Label the Tablets to indicate that the cefixime contained therein is in the trihydrate form.



• **USP REFERENCE STANDARDS** (11)  
 • USP Cefixime RS

## Cefmenoxime for Injection

» Cefmenoxime for Injection contains an amount of Cefmenoxime Hydrochloride equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ). It may contain Sodium Carbonate.

### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

**USP Reference standards** (11)—  
 USP Cefmenoxime Hydrochloride RS

### Identification—

**A: Ultraviolet Absorption** (197U)—

**Solution:** 25 µg per mL.

**Medium:** pH 6.8 buffer prepared as directed in the Assay under Cefmenoxime Hydrochloride.

**B:** The retention time of the cefmenoxime peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, both relative to the internal standard, as obtained in the Assay.

**Pyrogen** (151)—It meets the requirements, the test dose being 1.0 mL per kg of a solution of Cefmenoxime for Injection in sterile water for injection having a concentration of 60 mg of cefmenoxime per mL.

**Sterility Tests** (71)—It meets the requirements when tested as directed for Membrane Filtration under Test for Sterility of the Product to be Examined.

**pH** (791): between 6.4 and 7.9, in a solution containing the equivalent of 100 mg of cefmenoxime per mL.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 1.5% of its weight.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

### Assay—

pH 6.8 buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system—Prepare as directed in the Assay under Cefmenoxime Hydrochloride.

**Assay preparation 1** (where it is represented as being in a single-dose container)—Constitute a container of Cefmenoxime for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with water to obtain a solution containing the equivalent of about 1 mg of cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ) per mL. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, add 20.0 mL of Internal standard solution, dilute with Mobile phase to volume, and mix. This solution contains the equivalent of about 80 µg of cefmenoxime per mL.

**Assay preparation 2** (where the label states the quantity of cefmenoxime in a given volume of constituted solution)—Constitute a container of Cefmenoxime for Injection in a volume of water, accurately measured, equivalent to the volume of diluent specified in the labeling. Quantitatively dilute an accurately measured volume of the consti-

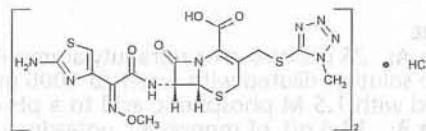
tuted solution with water to obtain a solution containing about 1 mg of cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ) per mL. Transfer 4.0 mL of this solution to a 50-mL volumetric flask, add 20.0 mL of Internal standard solution, dilute with Mobile phase to volume, and mix. This solution contains the equivalent of about 80 µg of cefmenoxime per mL.

**Procedure**—Proceed as directed for Procedure in the Assay under Cefmenoxime Hydrochloride. Calculate the quantity, in mg, of cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ) withdrawn from the container or in the portion of constituted solution taken by the formula:

$$1.6(L/D)(W_5 P_5 / 1000)(R_U / R_S)$$

in which *L* is the labeled quantity, in mg, of cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ) in the container or in the volume of constituted solution taken; *D* is the concentration, in µg of cefmenoxime per mL, of Assay preparation 1 or Assay preparation 2, based on the labeled quantity in the container or in the volume of constituted solution taken, respectively, and the extent of dilution; *W*<sub>5</sub> is the weight, in mg, of USP Cefmenoxime Hydrochloride RS taken to prepare the Standard preparation; *P*<sub>5</sub> is the designated cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ) content, in µg per mg, of USP Cefmenoxime Hydrochloride RS; and *R*<sub>U</sub> and *R*<sub>S</sub> are the response ratios of the cefmenoxime peak to the internal standard peak obtained from the Assay preparation and the Standard preparation, respectively.

## Cefmenoxime Hydrochloride



( $C_{16}H_{17}N_9O_5S_3$ )<sub>2</sub> · HCl 1059.58

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, hydrochloride (2:1), [6R-[6α,7β(Z)]]-

(6R,7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 7<sup>2</sup>-(Z)-(O-methyloxime), hydrochloride (2:1) [75738-58-8].

» Cefmenoxime Hydrochloride contains the equivalent of not less than 869 µg and not more than 1015 µg of cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—  
 USP Cefmenoxime Hydrochloride RS

### Identification—

**A: Ultraviolet Absorption** (197U)—

**Solution:** 25 µg per mL.

**Medium:** pH 6.8 buffer (prepared as directed in the Assay).

**B:** The retention time of the cefmenoxime peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, both relative to the internal standard, as obtained in the Assay.



**Crystallinity** (695): meets the requirements.

**Pyrogen** (151)—Where the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements, the test dose being 1.0 mL per kg of a solution in pyrogen-free sodium carbonate solution (prepared by dissolving 14.0 g of sodium carbonate, previously heated at 170° for not less than 4 hours, in 1000 mL of sterile water for injection) containing 60 mg per mL.

**Sterility Tests** (71)—Where the label states that it is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use *Fluid A* to each 100 mL of which has been added 2.0 g of sodium carbonate previously sterilized by heating at 180° for 2 hours.

**Water Determination, Method I** (921): not more than 1.5%, the *Test Preparation* being prepared as directed for a hygroscopic specimen, except to use 20 mL of a mixture of formamide (previously dried over anhydrous sodium sulfate for 24 hours) and methanol (2:1), instead of methanol, to dissolve the specimen, to use two 5-mL portions of the same formamide and methanol mixture to rinse the container, and to determine the water content of the formamide and methanol mixture.

#### Assay—

**pH 6.8 buffer**—Dissolve 6.4 g of monobasic potassium phosphate and 18.9 g of dibasic sodium phosphate in 750 mL of water, adjust with 1 N sodium hydroxide to a pH of 6.8 ± 0.1, dilute with water to 1000 mL, and mix.

**Mobile phase**—Prepare a suitable mixture of water, acetonitrile, and glacial acetic acid (50:10:1). Filter through a suitable filter of 0.5 µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of phthalimide in methanol containing 1.5 mg per mL.

**Standard preparation**—Transfer about 50 mg of USP Cefmenoxime Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask, add 10 mL of pH 6.8 buffer, and dissolve by swirling. Dilute with *Mobile phase* to volume, and mix. Transfer 4.0 mL of this solution to a second 50-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 80 µg of cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ) per mL.

**Assay preparation**—Transfer about 50 mg of Cefmenoxime Hydrochloride, accurately weighed, to a 50-mL volumetric flask, add 10 mL of pH 6.8 buffer, and dissolve by swirling. Dilute with *Mobile phase* to volume, and mix. Transfer 4.0 mL of this solution to a second 50-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 15-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the phthalimide and the cefmenoxime peaks is not less than 2.3; the column efficiency, determined from the cefmenoxime peak, is not less than 1200 theoretical plates when calculated by the formula:

$$5.545(t_r / W_{h/2})^2$$

the tailing factor for the cefmenoxime peak is not more than 1.6; and the relative standard deviation of replicate injections is not more than 2.0%.

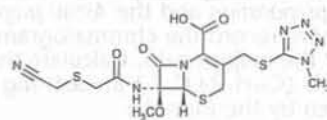
**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and meas-

ure the responses for the major peaks. Calculate the quantity, in µg, of cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ) in each mg of the Cefmenoxime Hydrochloride taken by the formula:

$$(W_S P_S / W_U)(R_U / R_S)$$

in which  $W_S$  is the weight, in mg, of USP Cefmenoxime Hydrochloride RS taken to prepare the *Standard preparation*;  $P_S$  is the designated cefmenoxime ( $C_{16}H_{17}N_9O_5S_3$ ) content, in µg per mg, of USP Cefmenoxime Hydrochloride RS;  $W_U$  is the weight, in mg, of Cefmenoxime Hydrochloride taken to prepare the *Assay preparation*, and  $R_U$  and  $R_S$  are the peak response ratios of the cefmenoxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefmetazole



$C_{15}H_{17}N_7O_5S_3$  471.53

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(cyanomethyl)thio]acetyl]amino]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, (6*R*-cis)-, (6*R*,7*S*)-7-[2-[(Cyanomethyl)thio]acetamido]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [56796-20-4].

» Cefmetazole contains not less than 970 µg and not more than 1030 µg of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cefmetazole RS

#### Identification—

A: *Infrared Absorption* (197M).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Water Determination, Method I** (921): not more than 0.5%.

#### Assay—

**Mobile phase**—Dissolve 5.75 g of monobasic ammonium phosphate in 700 mL of water, add 3.2 mL of a 40% solution of tetrabutylammonium hydroxide, 280 mL of methanol, and 25 mL of tetrahydrofuran, and mix. Adjust with phosphoric acid to a pH of 4.5 ± 0.1, pass through a filter having a 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Cefmetazole RS in *Mobile phase* to obtain a solution having a known concentration of about 200 µg of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) per mL. [NOTE—Use this solution within 10 minutes.]

**Resolution solution**—Prepare a solution of USP Cefmetazole RS in 0.01 N sodium hydroxide containing about 1 mg per mL. Heat at 95° for 10 minutes. To 1 mL of this solution add 2 mL of *Standard preparation*, and dilute with *Mobile phase* to obtain 20 mL of solution. This solution



contains cefmetazole and cefmetazole lactone (resolution compound).

**Assay preparation**—Transfer about 20 mg of Cefmetazole, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Use this solution within 10 minutes.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between cefmetazole and cefmetazole lactone is not less than 3.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 1250 theoretical plates; the tailing factor is not less than 0.94 and not more than 1.6; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in  $\mu$ g, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) in each mg of Cefmetazole taken by the formula:

$$100(C / M)(r_U / r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) in the *Standard preparation*;  $M$  is the quantity, in mg, of Cefmetazole taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the cefmetazole peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefmetazole Injection

» Cefmetazole Injection is a sterile isoosmotic solution of Cefmetazole and Sodium Citrate in Water for Injection. It contains one or more buffer substances and a tonicity-adjusting agent. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ).

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes the conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—

USP Cefmetazole RS  
USP Endotoxin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that observed in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of cefmetazole.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*, except to use water instead of *Fluid A*.

**pH** (791): between 4.2 and 6.2.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Assay**—

*Mobile phase*, *Resolution solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cefmetazole*.

**Assay preparation**—Allow the contents of a container of Injection to thaw, and mix the resultant solution. Transfer an accurately measured volume of this solution, equivalent to about 40 mg of cefmetazole, to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Use this solution within 10 minutes.]

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Cefmetazole*. Calculate the quantity, in mg, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) in each mL of the Injection by the formula:

$$0.2(C / VM)(r_U / r_S)$$

in which  $V$  is the volume, in mL, of Injection taken to prepare the *Assay preparation*, and the other terms are as defined therein.

## Cefmetazole for Injection

» Cefmetazole for Injection contains an amount of Cefmetazole Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ).

**Change to read:**

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for constitution* (CN 1-May-2017).

**USP Reference standards** (11)—

USP Cefmetazole RS  
USP Endotoxin RS

**Bacterial Endotoxins Test** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of cefmetazole.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements in the tests for *Identification*, *pH*, and *Water under Cefmetazole Sodium*. It meets also the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

**Assay**—

*Mobile phase*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cefmetazole*.

**Assay preparation 1** (where it is represented as being in a single-dose container)—Constitute Cefmetazole for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Mobile phase* to obtain a solution containing about 0.2 mg of cefmetazole per mL. [NOTE—Use this solution within 10 minutes.]



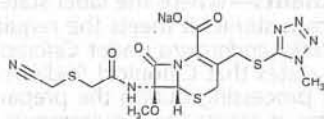
**Assay preparation 2** (where the label states the quantity of cefmetazole in a given volume of constituted solution)—Constitute Cefmetazole for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Mobile phase* to obtain a solution containing about 0.2 mg of cefmetazole per mL. [NOTE—Use this solution within 10 minutes.]

**Procedure**—Proceed as directed in the Assay under Cefmetazole. Calculate the quantity, in mg, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C/1000)(r_U / r_S)$$

in which *L* is the labeled quantity, in mg, of cefmetazole in the container, or in the volume of constituted solution taken; *D* is the concentration, in mg per mL, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) per mL, of Assay preparation 1 or Assay preparation 2, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively; *C* is the concentration, in µg per mL, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) in the Standard preparation; and *r<sub>U</sub>* and *r<sub>S</sub>* are the cefmetazole peak responses obtained from the relevant Assay preparation and the Standard preparation, respectively.

## Cefmetazole Sodium



$C_{15}H_{16}N_7NaO_5S_3$  493.52

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(cyanomethyl)thio]acetyl]amino]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, monosodium salt, (6*R*-*cis*)-.

Sodium (6*R*,7*S*)-7-[2-[(cyanomethyl)thio]acetamido]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [56796-39-5].

» Cefmetazole Sodium contains the equivalent of not less than 860 µg and not more than 1003 µg of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cefmetazole RS

USP Endotoxin RS

**Identification**—

A: Infrared Absorption (197M).

B: The retention time of the major peak in the chromatogram of the Assay preparation corresponds to that in the chromatogram of the Standard preparation, as obtained in the Assay.

**pH** (791): between 4.2 and 6.2, in a solution (1 in 10).

**Water Determination, Method I** (921): not more than 0.5%.

**Other requirements**—Where the label states that Cefmetazole Sodium is sterile, it meets the requirements in

the tests for *Sterility Tests* (71) and for *Bacterial endotoxins* under Cefmetazole for Injection. Where the label states that Cefmetazole Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements in the test for *Bacterial endotoxins* under Cefmetazole for Injection.

**Assay**—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under Cefmetazole.

**Assay preparation**—Transfer about 21 mg of Cefmetazole Sodium, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. [NOTE—Use this solution within 10 minutes.]

**Procedure**—Proceed as directed in the Assay under Cefmetazole. Calculate the quantity, in µg, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) per mg of Cefmetazole Sodium taken by the formula:

$$100(C/M)(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of cefmetazole ( $C_{15}H_{17}N_7O_5S_3$ ) in the Standard preparation; *M* is the quantity, in mg, of Cefmetazole Sodium taken to prepare the Assay preparation; and *r<sub>U</sub>* and *r<sub>S</sub>* are the cefmetazole peak responses obtained from the Assay preparation and the Standard preparation, respectively.

## Cefonicid for Injection

» Cefonicid for Injection contains an amount of Cefonicid Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefonicid ( $C_{18}H_{18}N_6O_8S_3$ ).

**Change to read:**

**Packaging and storage**—Preserve as described in **•Packaging and Storage Requirements** (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

**USP Reference standards** (11)—

USP Cefonicid Sodium RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.35 USP Endotoxin Unit per mg of cefonicid.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It responds to the *Identification tests* and meets the requirements for *Specific rotation, pH, and Water* under Cefonicid Sodium. It meets also the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

**Assay**—

*Mobile phase*—Prepare a mixture of water, methanol, and 0.2 M monobasic ammonium phosphate (33:5:3). Pass through a filter having a 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under Chromatography (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Cefonicid Sodium RS in *Mobile phase* to



obtain a solution having a known concentration of about 200 µg of cefonicid ( $C_{18}H_{18}N_6O_8S_3$ ) per mL.

**Assay preparation 1** (where it is represented as being in a single-dose container)—Constitute Cefonicid for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Mobile phase* to obtain a solution containing about 200 µg of cefonicid per mL.

**Assay preparation 2** (where the label states the quantity of cefonicid in a given volume of constituted solution)—Constitute Cefonicid for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Mobile phase* to obtain a solution containing about 200 µg of cefonicid per mL.

**Resolution solution**—Dissolve a quantity of USP Cefonicid Sodium RS in *Mobile phase* to obtain a solution containing about 0.2 mg per mL. Heat on a steam bath for 30 minutes, and cool. This *Resolution solution* contains a mixture of cefonicid and desacetyl cefonicid.

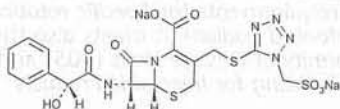
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the cefonicid peak and the desacetyl cefonicid peak is not less than 1.1; the column efficiency determined from the analyte peak is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cefonicid ( $C_{18}H_{18}N_6O_8S_3$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C)(r_U/r_S)$$

in which  $L$  is the labeled quantity, in mg, of cefonicid in the container, or in the volume of constituted solution taken;  $D$  is the concentration, in µg per mL, of cefonicid in *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution;  $C$  is the concentration, in µg per mL, of cefonicid in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the relevant *Assay preparation* and the *Standard preparation*, respectively.

## Cefonicid Sodium



$C_{18}H_{16}N_6Na_2O_8S_3$  586.53  
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(hydroxyphenylacetyl)amino]-8-oxo-3-[[[1-(sulfomethyl)-1H-tetrazol-5-yl]thio]methyl]disodium salt, [6R-[6α, 7β(R\*)]]-

(6R,7R)-[7-[(R)-Mandelamido]-8-oxo-3-[[[1-(sulfomethyl)-1H-tetrazol-5-yl]thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, disodium salt [61270-78-8].

» Cefonicid Sodium contains the equivalent of not less than 832 µg and not more than 970 µg of cefonicid ( $C_{18}H_{18}N_6O_8S_3$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cefonicid Sodium RS

USP Endotoxin RS

**Identification**—

**A:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefonicid, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** It responds to the tests for *Sodium* (191).

**Specific rotation** (781S): between  $-37^\circ$  and  $-47^\circ$ .

**Test solution:** 10 mg per mL, in methanol.

**pH** (791): between 3.5 and 6.5, in a solution (1 in 20).

**Water Determination, Method I** (921): not more than 5.0%.

**Other requirements**—Where the label states that Cefonicid Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cefonicid for Injection*. Where the label states that Cefonicid Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cefonicid for Injection*.

**Assay**—

**Mobile phase**—Prepare a mixture of water, methanol, and 0.2 M monobasic ammonium phosphate (33:5:2). Pass through a filter having a 0.5-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cefonicid Sodium RS in *Mobile phase* to obtain a solution having a known concentration of about 200 µg of cefonicid ( $C_{18}H_{18}N_6O_8S_3$ ) per mL.

**Assay preparation**—Transfer about 40 mg of Cefonicid Sodium, accurately weighed, to a 200-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Resolution solution**—Dissolve a quantity of USP Cefonicid Sodium RS in *Mobile phase* to obtain a solution containing about 0.2 mg per mL. Heat on a steam bath for 30 minutes, and cool. This *Resolution solution* contains a mixture of cefonicid and desacetyl cefonicid.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation* and the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the cefonicid and the desacetyl cefonicid peaks is not less than 1.1; the column efficiency determined from the analyte peak is not less than 1500 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; and the relative standard deviation for replicate injections of the *Standard preparation* is not more than 2%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-



tity, in  $\mu\text{g}$ , of cefonicid ( $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_8\text{S}_3$ ) per mg of the Cefonicid Sodium taken by the formula:

$$200(C/M)(r_U/r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL of cefonicid ( $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_8\text{S}_3$ ) in the *Standard preparation*;  $M$  is the quantity, in mg, of Cefonicid Sodium taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefoperazone Injection

» Cefoperazone Injection is a sterile solution of Cefoperazone Sodium and a suitable osmolality adjusting substance in Water for Injection. It may contain a suitable buffer. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefoperazone ( $\text{C}_{25}\text{H}_{27}\text{N}_9\text{O}_8\text{S}_2$ ).

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

### USP Reference standards (11)—

USP Cefoperazone Dihydrate RS  
USP Endotoxin RS

**Identification**—The retention time of the major peak for cefoperazone in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of cefoperazone.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.5 and 6.5.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

### Assay—

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Cefoperazone Sodium*.

*Assay preparation*—Quantitatively dilute an accurately measured volume of Injection with *Mobile phase* to obtain a solution containing about 0.16 mg of cefoperazone per mL.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Cefoperazone Sodium*. Calculate the quantity, in mg, of cefoperazone ( $\text{C}_{25}\text{H}_{27}\text{N}_9\text{O}_8\text{S}_2$ ), in the volume of Injection taken by the formula:

$$(L/D)(C)(r_U/r_S)$$

in which  $L$  is the labeled quantity, in mg, of cefoperazone ( $\text{C}_{25}\text{H}_{27}\text{N}_9\text{O}_8\text{S}_2$ ), in the volume of Injection taken;  $D$  is the concentration, in mg of cefoperazone ( $\text{C}_{25}\text{H}_{27}\text{N}_9\text{O}_8\text{S}_2$ ) per mL, of the *Assay preparation*, based on the labeled quantity in the portion of Injection taken and the extent of dilution; and the other terms are as defined therein.

## Cefoperazone for Injection

» Cefoperazone for Injection contains an amount of Cefoperazone Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefoperazone ( $\text{C}_{25}\text{H}_{27}\text{N}_9\text{O}_8\text{S}_2$ ).

### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for constitution* (CN 1-May-2017).

### USP Reference standards (11)—

USP Cefoperazone Dihydrate RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of cefoperazone.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.5 and 6.5, in a solution (1 in 4).

**Water Determination, Method I** (921): not more than 5.0%, except that where it is in the freeze-dried form, the limit is not more than 2.0%.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for the *Identification tests* under *Cefoperazone Sodium* and meets the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

### Assay—

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Cefoperazone Sodium*.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Cefoperazone for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Mobile phase* to obtain a solution containing about 0.16 mg of cefoperazone per mL.

*Assay preparation 2* (where the label states the quantity of cefoperazone in a given volume of constituted solution)—Constitute Cefoperazone for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Mobile phase* to obtain a solution containing about 0.16 mg of cefoperazone per mL.

*Procedure*—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu\text{g}$  of cefoperazone per mg, of the Cefoperazone for Injection taken by the formula:

$$1000(C/M)(r_U/r_S)$$

in which  $C$  is the concentration, in mg of cefoperazone ( $\text{C}_{25}\text{H}_{27}\text{N}_9\text{O}_8\text{S}_2$ ) per mL, of the *Standard preparation*;  $M$  is the concentration, in mg per mL, of the *Assay preparation*, based

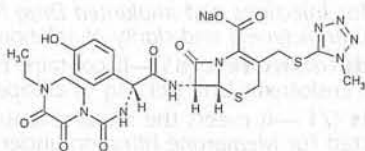


extent of dilution; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the quantity, in mg, of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ), withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C)(r_U/r_S)$$

in which  $L$  is the labeled quantity, in mg, of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ), in the container, or in the volume of constituted solution taken; and  $D$  is the concentration, in mg of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ) per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; and the other terms are as defined therein.

## Cefoperazone Sodium



$C_{25}H_{26}N_9NaO_8S_2$  667.65

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[4-ethyl-2,3-dioxo-1-piperazinyl]carbonyl]amino](4-hydroxyphenyl)acetyl]amino]-3-[[[1-methyl-1H-tetrazol-5-yl]thio]methyl]-8-oxo-, monosodium salt, [6R-[6 $\alpha$ ,7 $\beta$ (R\*)]]-. Sodium (6R,7R)-7-[(R)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-2-(p-hydroxyphenyl)acetamido]-3-[[[1-methyl-1H-tetrazol-5-yl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [62893-20-3].

» Cefoperazone Sodium contains the equivalent of not less than 870  $\mu$ g and not more than 1015  $\mu$ g of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cefoperazone Dihydrate RS

USP Endotoxin RS

**Identification**—

A: The retention time of the major peak for cefoperazone in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

B: It responds to the tests for *Sodium* (191).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 4.5 and 6.5, in a solution (1 in 4).

**Water Determination, Method I** (921): not more than 5.0%.

**Other requirements**—Where the label states that Cefoperazone Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cefoperazone for Injection*. Where the label states that Cefoperazone Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements

## Assay—

**Mobile phase**—Place 14 mL of triethylamine and 5.7 mL of glacial acetic acid in a 100-mL volumetric flask, dilute with water to volume, and mix. Prepare a suitable mixture of water, acetonitrile, 1 N acetic acid, and this solution (876:120:2.8:1.2). Filter through a membrane filter (1- $\mu$ m or finer porosity), and degas.

**Standard preparation**—Dissolve a suitable quantity of USP Cefoperazone Dihydrate RS, accurately weighed, in *Mobile phase* to obtain a solution having a known concentration of about 0.16 mg of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ) per mL.

**Assay preparation**—Using a suitable quantity of Cefoperazone Sodium, accurately weighed, proceed as directed under *Standard preparation*.

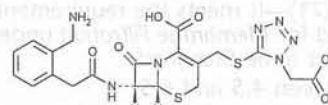
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%, and the tailing factor is not more than 1.5.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g of cefoperazone per mg, of the Cefoperazone Sodium taken by the formula:

$$1000(C/M)(r_U/r_S)$$

in which  $C$  is the concentration, in mg of cefoperazone ( $C_{25}H_{27}N_9O_8S_2$ ) per mL, of the *Standard preparation*;  $M$  is the concentration, in mg per mL, of the *Assay preparation* based on the weight of Cefoperazone Sodium taken and the extent of dilution; and  $r_U$  and  $r_S$  are the peak responses from the *Assay preparation* and the *Standard preparation*, respectively.

## Ceforanide



$C_{20}H_{21}N_7O_6S_2$  519.55

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[2-(aminomethyl)phenyl]acetyl]amino]-3-[[[1-(carboxymethyl)-1H-tetrazol-5-yl]thio]methyl]-8-oxo-, (6R-trans)-, (6R,7R)-7-[2-( $\alpha$ -Amino-o-tolyl)acetamido]-3-[[[1-(carboxymethyl)-1H-tetrazol-5-yl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[o-(Aminomethyl)phenylacetamido]-3-[[[1-(carboxymethyl)-1H-tetrazol-5-yl]thio]methyl]-3-cephem-4-carboxylic acid [60925-61-3].

» Ceforanide contains not less than 900  $\mu$ g and not more than 1050  $\mu$ g of ceforanide ( $C_{20}H_{21}N_7O_6S_2$ ) per mg.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—



**Identification—**

**A:** *Infrared Absorption* (197M).

**B:** The retention time of the major peak for ceforanide in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial Endotoxins Test** (85)—Where the label states that Ceforanide is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains not more than 0.25 USP Endotoxin Unit per mg of ceforanide.

**Sterility Tests** (71)—Where the label states that Ceforanide is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to dissolve 6 g of Ceforanide in *Fluid A* to each 1000 mL of which has been added 10 g of sterile L-lysine, and to rinse the membrane with three 100-mL portions of *Fluid D* and one 100-mL portion of *Fluid A*.

**pH** (791): between 2.5 and 4.5, in a suspension containing 50 mg per mL.

**Water Determination, Method I** (921): not more than 5.0%.

**Assay—**

**Mobile phase**—Mix 18 mL of tetrabutylammonium hydroxide solution (1 in 10) and 8.6 mL of 11 N potassium hydroxide, and add the mixture to 700 mL of water. Add 200 mL of methanol, adjust with phosphoric acid to a pH of 7.0, and add water to obtain 1000 mL of solution, making adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Filter, using a filter having a porosity of 1  $\mu$ m or finer, and degas.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ceforanide RS in *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL. Use this solution within 5 minutes.

**Assay preparation**—Using a suitable quantity of Ceforanide, accurately weighed, proceed as directed under *Standard preparation*. Use this solution within 5 minutes.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm  $\times$  30-cm column that contains 5- to 10- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 1900 theoretical plates; the tailing factor for the analyte peak is not more than 1.2; the capacity factor,  $k'$ , is not less than 1.8 and not more than 5.0; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of  $C_{20}H_{21}N_7O_6S_2$  in each mg of the Ceforanide taken by the formula:

$$(CP/M)(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Ceforanide RS in the *Standard preparation*;  $P$  is the potency, in  $\mu$ g per mg, of the USP Ceforanide RS;  $M$  is the concentration, in mg per mL, of the *Assay preparation*, based on the amount of Ceforanide taken and the extent of dilution; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Ceforanide for Injection**

» Ceforanide for Injection is a sterile mixture of Ceforanide and L-lysine. It contains not less than 900  $\mu$ g and not more than 1050  $\mu$ g of ceforanide ( $C_{20}H_{21}N_7O_6S_2$ ) per mg on the L-lysine-free basis, and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{20}H_{21}N_7O_6S_2$ .

**Change to read:**

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

**USP Reference standards** (11)—

USP Ceforanide RS

USP Endotoxin RS

**Identification—**

**A:** The retention time of the major peak for L-lysine in the chromatogram of the *Test preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the test for *Content of L-lysine*.

**B:** The retention time of the major peak in ceforanide in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*; as obtained in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.25 USP Endotoxin Unit per mg of ceforanide.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to constitute each container with 3 mL of *Fluid A* for each g of ceforanide contained therein, and to rinse the membrane with three 100-mL portions of *Fluid D* and one 100-mL portion of *Fluid A*.

**pH** (791): between 5.5 and 8.5, when constituted as directed in the labeling.

**Water Determination, Method I** (921): not more than 3.0%.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Content of L-lysine—**

**Mobile phase**—Mix 62 volumes of methanol and 38 volumes of water, and adjust with glacial acetic acid to a pH of 3.0, making adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Stock standard solution**—Transfer about 36 mg of L-lysine, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Standard preparation**—Transfer 2.0 mL of *Stock standard solution* to a glass-stoppered, 10-mL volumetric flask, add 2.0 mL of a 1.4% solution of tris(hydroxymethyl)aminomethane and 3.0 mL of a 1.5% solution of 1-fluoro-2,4-dinitrobenzene in dehydrated alcohol, insert the stopper tightly, and mix. Heat at 50° in a water bath for 30 minutes. Remove the flask from the water bath, allow to cool, dilute with methanol to volume, and mix.

**Test preparation**—Transfer about 150 mg of Ceforanide for Injection, accurately weighed, to a 100-mL volumetric flask, add water to volume, and mix. Transfer 2.0 mL of the resulting solution to a glass-stoppered, 10-mL volumetric flask, and proceed as directed under *Standard preparation*, beginning with "add 2.0 mL of a 1.4% solution of tris(hydroxymethyl)aminomethane."



**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5- to 10-μm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the derivatized L-lysine peak is not less than 1500 theoretical plates; the tailing factor for the same peak is not more than 1.3, the resolution,  $R_s$ , between the derivatized L-lysine peak and the 1-fluoro-2,4-dinitrobenzene peak is not less than 4.5; the capacity factor,  $k'$ , is not less than 4 and not more than 6; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of L-lysine in the Ceforanide for Injection taken by the formula:

$$10(C/M)(r_U/r_S)$$

in which  $C$  is the concentration, in μg per mL, of L-lysine in the *Stock standard solution*;  $M$  is the quantity, in mg, of Ceforanide for Injection taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively. Use this percentage to calculate, on an L-lysine-free basis, the result from *Assay preparation 1* obtained as directed in the *Assay*.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

#### Assay—

**Mobile phase, Standard preparation, and Chromatographic system**—Prepare as directed in the *Assay* under Ceforanide.

**Assay preparation 1**—Dissolve a suitable quantity of Ceforanide for Injection, accurately weighed, in *Mobile phase*, and dilute quantitatively and stepwise with *Mobile phase* to obtain a solution having a concentration of about 1 mg of ceforanide per mL. Use this solution within 5 minutes.

**Assay preparation 2** (where it is represented as being in a single-dose container)—Constitute Ceforanide for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively and stepwise with *Mobile phase* to obtain a solution containing about 1 mg of ceforanide per mL. Use this solution within 5 minutes.

**Assay preparation 3** (where the label states the quantity of ceforanide in a given volume of constituted solution)—Constitute Ceforanide for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively and stepwise with *Mobile phase* to obtain a solution containing about 1 mg of ceforanide per mL. Use this solution within 5 minutes.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under Ceforanide. Calculate the quantity, in μg, of ceforanide ( $C_{20}H_{21}N_7O_6S_2$ ) in each mg of the Ceforanide for Injection taken by the formula:

$$(CP/M)(r_U/r_S)$$

in which  $M$  is the concentration, in mg per mL, of *Assay preparation 1* based on the weight of Ceforanide for Injection taken and the extent of dilution, and the other terms are as defined therein. Calculate the quantity, in mg, of

$C_{20}H_{21}N_7O_6S_2$  withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(CP/1000)(r_U/r_S)$$

in which  $L$  is the labeled quantity, in mg, of ceforanide in the container, or in the volume of constituted solution taken;  $D$  is the concentration, in mg of ceforanide per mL, of *Assay preparation 2* or *Assay preparation 3*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively; and the extent of dilution, and the other terms are as defined therein.

## Cefotaxime Injection

### DEFINITION

Cefotaxime Injection is a sterile solution of Cefotaxime Sodium in Water for Injection. It contains one or more suitable buffers, and it may contain Dextrose or Sodium Chloride as a tonicity-adjusting agent. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ).

### IDENTIFICATION

- A.** The retention time of the major peak in the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Buffer:** 7.1 g/L of anhydrous dibasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 6.25

**Solution A:** Methanol and *Buffer* (14:86). Pass through a filter having a pore size of 0.5 μm or less, and degas before use.

**Solution B:** Methanol and *Buffer* (40:60). Pass through a filter having a pore size of 0.5 μm or less, and degas before use.

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	100	0
9	80	20
16	80	20
46	0	100
51	0	100
56	100	0

**Standard solution:** 0.8 mg/mL of USP Cefotaxime Sodium RS in *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.

**System suitability solution:** Mix 1 mL of *Standard solution*, 7.0 mL of water, and 2.0 mL of methanol. Add 25 mg of sodium carbonate, mix, and allow to stand at room temperature for 10 min, with occasional swirling. Add 3 drops of glacial acetic acid and 1 mL of *Standard solution*.

**Sensitivity solution:** 1.6 μg/mL of USP Cefotaxime Sodium RS in *Solution A*.

**Sample solution:** Nominally 0.8 mg/mL of cefotaxime, prepared as follows. Allow one container of Injection to thaw, and mix. Transfer an aliquot of the Injection to a suitable volumetric flask, and dilute with *Solution A* to volume.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 3.9-mm × 15-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 μL

**System suitability**

**Samples:** *Standard solution*, *System suitability solution*, and *Sensitivity solution*. [NOTE—The retention times for desacetylcefotaxime and cefotaxime in the *System suitability solution* are 3.5 min and 14 min, respectively. The retention time for cefotaxime in the *Standard solution* is 12–15 min.]

**Suitability requirements**

**Sensitivity:** The response of the cefotaxime peak from the *Sensitivity solution* is between 0.18% and 0.22% of the response of the cefotaxime peak of the *Standard solution*.

**Resolution:** NLT 20 between desacetylcefotaxime and cefotaxime, *System suitability solution*

**Tailing factor:** NMT 2, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of cefotaxime (C<sub>16</sub>H<sub>17</sub>N<sub>5</sub>O<sub>7</sub>S<sub>2</sub>) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak area of the *Sample solution*

$r_S$  = peak area of the *Standard solution*

$C_S$  = concentration of USP Cefotaxime Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefotaxime in the *Sample solution* (mg/mL)

$P$  = potency of cefotaxime in USP Cefotaxime Sodium RS (μg/mg)

Acceptance criteria: 90.0%–110.0%

**IMPURITIES****• ORGANIC IMPURITIES**

Mobile phase, *Standard solution*, *System suitability solution*, *Sensitivity solution*, *Sample solution*, *Chromatographic system*, and *System suitability*: Proceed as directed in the Assay.

**Analysis**

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = r_U/(r_T + r_C) \times 100$$

$r_U$  = peak area of each individual impurity

$r_T$  = sum of all of the impurity peak areas

$r_C$  = peak area of cefotaxime

**Acceptance criteria**

Disregard any impurity peak that is less than 0.1%.

Individual impurities: NMT 6.0%

Total impurities: NMT 10.0%

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.20 USP Endotoxin Unit/mg of cefotaxime
- **STERILITY TESTS** (71): It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.
- **PH** (791): 5.0–7.5
- **PARTICULATE MATTER IN INJECTIONS** (788): It meets the requirements for small-volume injections.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, as described in *Packaging and Storage Requirements* (659). Maintain in the frozen state.
- **LABELING:** It meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just before use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- **USP REFERENCE STANDARDS** (11)
  - USP Cefotaxime Sodium RS
  - USP Endotoxin RS

**Cefotaxime for Injection****DEFINITION**

Cefotaxime for Injection contains an amount of Cefotaxime Sodium equivalent to NLT 90.0% and NMT 115.0% of the labeled amount of cefotaxime (C<sub>16</sub>H<sub>17</sub>N<sub>5</sub>O<sub>7</sub>S<sub>2</sub>).

**IDENTIFICATION**

Where the label indicates that there are no added substances:

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL, Sodium** (191): Meets the requirements

Where the label indicates that there are added substances:

- **C.** The retention time of the major peak of the *Sample solution* 1, 2, 3, or 4 corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****• PROCEDURE**

**Buffer:** 7.1 g/L of anhydrous dibasic sodium phosphate in water, adjusted with phosphoric acid to a pH of 6.25

**Solution A:** Methanol and *Buffer* (14:86). Pass through a filter having a pore size of 0.5 μm or finer, and degas before use.

**Solution B:** Methanol and *Buffer* (40:60). Pass through a filter having a pore size of 0.5 μm or finer, and degas before use.

Mobile phase: See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	100	0
9	80	20
16	80	20
46	0	100
51	0	100
56	100	0

**Standard solution:** 0.8 mg/mL of USP Cefotaxime Sodium RS in *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.

**System suitability solution:** Mix 1 mL of *Standard solution*, 7.0 mL of water, and 2.0 mL of methanol. Add 25 mg of sodium carbonate, mix, and allow to stand at room temperature for 10 min, with occasional swirling. Add 3 drops of glacial acetic acid and 1 mL of *Standard solution*.

**Sensitivity solution:** 1.6 μg/mL of USP Cefotaxime Sodium RS in *Solution A*

**Sample solution 1** (for use where the *Weight Variation* test is to be performed): 0.8 mg/mL of Cefotaxime for Injection in *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.



**Sample solution 2** (for use in assaying vials and infusion bottles packaged for dispensing): Nominally 0.8 mg/mL of cefotaxime, prepared as follows. Constitute one container of Cefotaxime for Injection with the smallest volume of diluent specified in the labeling. Invert the container, and withdraw all of the withdrawable contents of the container with a hypodermic needle and syringe. Transfer the contents of the syringe to a 100-mL volumetric flask, dilute with *Solution A* to volume, and mix. Do not rinse the syringe or container. Dilute a suitable aliquot of this solution with *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.

**Sample solution 3** (for use in assaying piggyback infusion bottles): Nominally 0.8 mg/mL of cefotaxime, prepared as follows. Constitute one container of Cefotaxime for Injection with the smallest volume of diluent recommended in the labeling, using the directions specified in the labeling. Invert the container, and withdraw all of the withdrawable contents of the container with a hypodermic needle and syringe. Transfer the contents of the syringe to a 100-mL volumetric flask, dilute with *Solution A* to volume, and mix. Do not rinse the syringe or container. Dilute a suitable aliquot of this solution with *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.

**Sample stock solution 4** (for use in assaying pharmacy bulk packages where the label states the quantity of cefotaxime in a given volume of constituted solution): Nominally 10 mg/mL of cefotaxime, prepared as follows. Constitute one container of Cefotaxime for Injection with the volume of diluent specified in the labeling. With a hypodermic needle and syringe, withdraw a suitable aliquot of the reconstituted product, transfer to a suitable volumetric flask, dilute with *Solution A* to volume, and mix. Do not rinse the syringe or container.

**Sample solution 4:** Nominally 0.8 mg/mL of cefotaxime from *Sample stock solution 4* in *Solution A*. Use this solution promptly. It may be used within 24 h if stored in a refrigerator.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 3.9-mm × 15-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 μL

#### System suitability

**Samples:** *Standard solution*, *System suitability solution*, and *Sensitivity solution*. [NOTE—The retention times for desacetylcefotaxime and cefotaxime in the *System suitability solution* are 3.5 min and 14 min, respectively. The retention time for cefotaxime in the *Standard solution* is 12–15 min.]

#### Suitability requirements

**Sensitivity:** The response of the cefotaxime peak from the *Sensitivity solution* is between 0.18% and 0.22% of the response of the cefotaxime peak of the *Standard solution*.

**Resolution:** NLT 20 between desacetylcefotaxime and cefotaxime, *System suitability solution*

**Tailing factor:** NMT 2, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

#### Analysis

**Samples:** *Sample solution 1*, or *Sample solution 2*, or *Sample solution 3*, or *Sample solution 4*, and *Standard solution*

Calculate the percentage of the labeled amount of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ) withdrawn from the con-

tainer, or in the portion of Cefotaxime for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak area of the *Sample solution*

$r_S$  = peak area of the *Standard solution*

$C_S$  = concentration of USP Cefotaxime Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefotaxime in *Sample solution 1, 2, 3, or 4* (mg/mL)

$P$  = potency of cefotaxime in USP Cefotaxime Sodium RS (μg/mg)

**Acceptance criteria:** 90.0%–115.0%. Where the test for *Uniformity of Dosage Units* has been performed using the *Procedure for Content Uniformity*, use the average of these determinations as the Assay value.

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

#### IMPURITIES

- **ORGANIC IMPURITIES**

Mobile phase; *Standard solution*; *System suitability solution*; *Sample solutions 1, 2, 3 or 4*; *Chromatographic system*; and *System suitability*: Proceed as directed in the Assay.

#### Analysis

Calculate the percentage of each impurity in the portion of Cefotaxime for Injection taken:

$$\text{Result} = r_U/(r_T + r_C) \times 100$$

$r_U$  = peak area of each individual impurity

$r_T$  = sum of all of the impurity peak areas

$r_C$  = peak area of cefotaxime

#### Acceptance criteria

Disregard any impurity peak that is less than 0.1%.

**Individual impurities:** NMT 6.0%

**Total impurities:** NMT 10.0%

#### SPECIFIC TESTS

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.20 USP Endotoxin Unit/mg of cefotaxime
- **STERILITY TESTS** (71): It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.
- **PARTICULATE MATTER IN INJECTIONS** (788): It meets the requirements for small-volume injections.
- **PH** (791)  
*Sample solution:* 100-mg/mL solution  
**Acceptance criteria:** 4.5–6.5
- **LOSS ON DRYING** (731)  
**Analysis:** Dry at 100°–105° for 3 h.  
**Acceptance criteria:** NMT 3.0%
- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*.

#### ADDITIONAL REQUIREMENTS

##### Change to read:

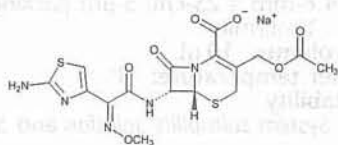
- **PACKAGING AND STORAGE:** • *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for constitution* (CN 1-May-2017).



• **USP REFERENCE STANDARDS** (11)

- USP Cefotaxime Sodium RS
- USP Endotoxin RS

## Cefotaxime Sodium



$C_{16}H_{16}N_5NaO_7S_2$  477.45

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyloxy)methyl]-7-[[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-, monosodium salt, [6R-[6 $\alpha$ ,7 $\beta$ (Z)]]-

Sodium (6R,7R)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 7 $\alpha$ -(Z)-(O-methyloxime), acetate (ester) [64485-93-4].

### DEFINITION

Cefotaxime Sodium contains the equivalent of NLT 916  $\mu$ g/mg and NMT 964  $\mu$ g/mg of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ), calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- C. IDENTIFICATION TESTS—GENERAL**, Sodium (191): It meets the requirements.

### ASSAY

#### • PROCEDURE

**Buffer:** 7.1 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 6.25.

**Solution A:** Methanol and *Buffer* (14:86)

**Solution B:** Methanol and *Buffer* (40:60)

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
7	100	0
9	80	20
16	80	20
45	0	100
50	0	100
55	100	0
65	100	0

**System suitability stock solution:** 0.08 mg/mL each of USP Cefetamet RS and USP Cefotaxime Related Compound E RS prepared as follows. Dissolve USP Cefetamet RS and USP Cefotaxime Related Compound E RS in the minimum volume of methanol. Sonicate, if necessary, and dilute with *Solution A* to volume.

**System suitability solution:** 8  $\mu$ g/mL each of cefetamet and cefotaxime related compound E from *System suitability stock solution* in *Solution A*. Store refrigerated, and use within 24 h.

**Standard solution:** 0.8 mg/mL of USP Cefotaxime Sodium RS in *Solution A*. Store refrigerated, and use within 24 h.

**Sensitivity solution:** 1.6  $\mu$ g/mL of USP Cefotaxime Sodium RS from the *Standard solution* in *Solution A*. Store refrigerated, and use within 24 h.

**Sample solution:** 0.8 mg/mL of Cefotaxime Sodium in *Solution A*. Store refrigerated, and use within 24 h.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 3.9-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Temperatures**

**Column:** 30°

**Autosampler:** 4°

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

### System suitability

**Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—See *Table 2* for relative retention times.]

### Suitability requirements

**Resolution:** NLT 1.5 between cefetamet and cefotaxime related compound E, *System suitability solution*

**Tailing factor:** NMT 2, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

**Sensitivity:** Calculate the peak response ratio:

$$\text{Result} = (r_U/r_S) \times 100$$

$r_U$  = peak response from the *Sensitivity solution*

$r_S$  = peak response from the *Standard solution*

**Acceptance criteria:** 0.18%–0.22%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in  $\mu$ g/mg, of cefotaxime ( $C_{16}H_{17}N_5O_7S_2$ ) in the portion of Cefotaxime Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cefotaxime Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cefotaxime Sodium in the *Sample solution* (mg/mL)

$P$  = potency of cefotaxime in USP Cefotaxime Sodium RS ( $\mu$ g/mg)

**Acceptance criteria:** 916–964  $\mu$ g/mg on the dried basis

### IMPURITIES

#### • ORGANIC IMPURITIES, PROCEDURE 1

Use *Organic Impurities, Procedure 1*, when the impurity profile includes *N*-formyl cefotaxime and cefotaxime dioxime.

**Buffer, Solution A, Solution B, Mobile phase, System suitability solution, Sensitivity solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Cefotaxime Sodium taken:

$$\text{Result} = [r_U/(r_T + r_C)] \times 100$$

$r_U$  = peak response of each individual impurity

$r_T$  = sum of all of the impurity peak responses

$r_C$  = peak response of cefotaxime

**Acceptance criteria:** See *Table 2*. The reporting threshold is 0.1%.



Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deacetylcefotaxime <sup>a</sup>	0.26	1.0
Cefetamet <sup>b</sup>	0.52	1.0
Cefotaxime related compound E <sup>c</sup>	0.62	1.0
Cefotaxime	1.0	—
N-Formyl cefotaxime <sup>d</sup>	1.8	1.0
E-Cefotaxime <sup>e</sup>	2.2	1.0
Cefotaxime dimer <sup>f</sup>	2.3	1.0
Cefotaxime dioxime <sup>g</sup>	3.0	0.2
Any individual unspecified impurity	—	0.2
Total impurities	—	3.0

<sup>a</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>b</sup> Deacetoxycefotaxime; (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>c</sup> Deacetylcefotaxime lactone; (Z)-2-(2-Aminothiazol-4-yl)-N-[(5aR,6R)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]-2-(methoxyimino)acetamide.

<sup>d</sup> (6R,7R)-3-(Acetoxymethyl)-7-[(Z)-2-(2-formamidothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>e</sup> (6R,7R)-3-(Acetoxymethyl)-7-[(E)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> (6R,7R)-3-[(4-[(Z)-2-[(6R,7R)-3-(Acetoxymethyl)-2-carboxy-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylamino]-1-(methoxyimino)-2-oxoethyl]thiazol-2-ylamino)methyl]-7-[(E)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>g</sup> (6R,7R)-3-(Acetoxymethyl)-7-[(Z)-2-[(Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]thiazol-4-yl]-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

#### • ORGANIC IMPURITIES, PROCEDURE 2

Use *Organic Impurities, Procedure 2*, when the impurity profile includes thiazolylglyoxalic methyloxime, 7-aminocephalosporanic acid, cefotaxime open ring lactone, and bromoacetyl analog.

**Buffer:** 3.6 g/L of anhydrous dibasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 6.2.

**Solution A:** Acetonitrile and *Buffer* (2:98)

**Solution B:** Acetonitrile and *Buffer* (60:40)

**Mobile phase:** See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
25	80	20
40	60	40
55	0	100
60	0	100
65	100	0
75	100	0

**Diluent:** 4.6 g/L of anhydrous dibasic sodium phosphate and 3.5 g/L of monobasic potassium phosphate in water

**System suitability stock solution:** 0.1 mg/mL of USP Cefotaxime Related Compound E RS prepared as follows. Dissolve in acetonitrile and *Diluent*, using 20% and 40% respectively of the final volume, sonicate as needed to dissolve, and dilute with *Diluent* to volume.

**System suitability solution:** 10 µg/mL of cefotaxime related compound E from *System suitability stock solution* and 1 mg/mL of USP Cefotaxime Sodium RS in *Diluent*. Store refrigerated, and use within 2 h.

**Standard solution:** 10 µg/mL of USP Cefotaxime Sodium RS in *Diluent*. Store refrigerated, and use within 2 h.

**Sample solution:** 1 mg/mL of Cefotaxime Sodium in *Diluent*. Store refrigerated, and use within 2 h.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 235 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

**Autosampler temperature:** 4°

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 4.0 between cefotaxime and cefotaxime related compound E, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cefotaxime Sodium taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response of each impurity from the *Sample solution*

$r_s$  = peak response of cefotaxime from the *Standard solution*

$C_s$  = concentration of USP Cefotaxime Sodium RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Cefotaxime Sodium in the *Sample solution* (mg/mL)

$P$  = potency of cefotaxime in USP Cefotaxime Sodium RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** See *Table 4*. The reporting threshold is 0.05%.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Thiazolylglyoxalic methyloxime <sup>a</sup>	0.13	0.15
7-Aminocephalosporanic acid <sup>b</sup>	0.41	0.15
Deacetylcefotaxime <sup>c</sup>	0.57	1.0

<sup>a</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.

<sup>b</sup> (6R,7R)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>c</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>d</sup> (2R)-2-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-(7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl)acetic acid. This system resolves two diastereoisomers.

<sup>e</sup> Deacetoxycefotaxime; (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> Deacetylcefotaxime lactone; (Z)-2-(2-Aminothiazol-4-yl)-N-[(5aR,6R)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]-2-(methoxyimino)acetamide.

<sup>g</sup> (6R,7R)-3-[(4-[(Z)-2-[(6R,7R)-3-(Acetoxymethyl)-2-carboxy-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylamino]-1-(methoxyimino)-2-oxoethyl]thiazol-2-ylamino)methyl]-7-[(E)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>h</sup> (6R,7R)-3-(Acetoxymethyl)-7-[(E)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>i</sup> (6R,7R)-3-(Acetoxymethyl)-7-[(Z)-4-bromo-2-(methoxyimino)-3-oxobutanamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.



Table 4 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
	0.60	0.15
Cefotaxime open ring lactone <sup>d</sup>	0.71	0.15
Cefetamet <sup>e</sup>	0.74	1.0
Cefotaxime	1.0	—
Cefotaxime related compound E <sup>f</sup>	1.08	1.0
Cefotaxime dimers <sup>g</sup>	1.26	1.0
E-Cefotaxime <sup>h</sup>	1.34	1.0
Bromoacetyl analog <sup>i</sup>	1.48	0.15
Any individual unspecified impurity	—	0.2
Total impurities	—	3.0

<sup>a</sup> (Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetic acid.

<sup>b</sup> (6R,7R)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>c</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>d</sup> (2R)-(Z)-2-[2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-2-(7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl)acetic acid. This system resolves two diastereoisomers.

<sup>e</sup> Deacetoxycefotaxime; (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> Deacetylcefotaxime lactone; (Z)-2-(2-Aminothiazol-4-yl)-N-[(5aR,6R)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]-2-(methoxyimino)acetamide.

<sup>g</sup> (6R,7R)-3-[(4-[(Z)-2-[(6R,7R)-3-(Acetoxymethyl)-2-carboxy-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-ylamino]-1-(methoxyimino)-2-oxoethyl]thiazol-2-ylamino)methyl]-7-[(E)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>h</sup> (6R,7R)-3-(Acetoxymethyl)-7-[(F)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>i</sup> (6R,7R)-3-(Acetoxymethyl)-7-[(Z)-4-bromo-2-(methoxyimino)-3-oxobutanamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

## SPECIFIC TESTS

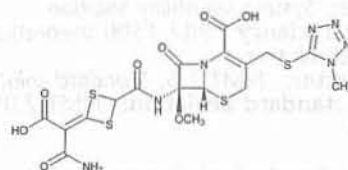
- **OPTICAL ROTATION, Specific Rotation (781S)**  
Sample solution: 10 mg/mL in water  
Acceptance criteria: +58° to +64°
- **pH (791)**  
Sample solution: 100 mg/mL in water  
Acceptance criteria: 4.5–6.5
- **LOSS ON DRYING (731)**  
Analysis: Dry at 100°–105° for 3 h.  
Acceptance criteria: NMT 3.0%
- **STERILITY TESTS (71):** Where the label states that Cefotaxime Sodium is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Cefotaxime Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.20 Endotoxin Units/mg of cefotaxime.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS (11)**  
USP Cefetamet RS  
Deacetoxycefotaxime; (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.  
C<sub>14</sub>H<sub>15</sub>N<sub>5</sub>O<sub>5</sub>S<sub>2</sub> 397.43

USP Cefotaxime Related Compound E RS  
Deacetylcefotaxime lactone; (Z)-2-(2-Aminothiazol-4-yl)-N-[(5aR,6R)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]-2-(methoxyimino)acetamide.  
C<sub>14</sub>H<sub>13</sub>N<sub>5</sub>O<sub>5</sub>S<sub>2</sub> 395.41  
USP Cefotaxime Sodium RS  
USP Endotoxin RS

## Cefotetan



C<sub>17</sub>H<sub>17</sub>N<sub>7</sub>O<sub>8</sub>S<sub>4</sub> 575.62  
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[4-(2-amino-1-carboxy-2-oxoethylidene)-1,3-dithietan-2-yl]carbonyl]amino]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, [6R-(6α,7α)-]; (6R,7S)-4-[[2-Carboxy-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl]carbonyl]-1,3-dithietane-Δ<sup>2,α</sup>-malonic acid; (6R,7S)-7-[4-(Carbamoylcarboxymethylene)-1,3-dithietane-2-carboxamido]-7-methoxy-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [69712-56-7].

## DEFINITION

Cefotetan contains NLT 950 μg/mg and NMT 1030 μg/mg of cefotetan (C<sub>17</sub>H<sub>17</sub>N<sub>7</sub>O<sub>8</sub>S<sub>4</sub>), calculated on the anhydrous basis.

## IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

- **PROCEDURE**  
Protect the *Standard solution*, the *System suitability solution*, and the *Sample solution* from light, and use within 2 h.  
**Mobile phase:** Methanol, acetonitrile, glacial acetic acid, and 0.1 M phosphoric acid (105:105:100:1700)  
**Standard solution:** 0.2 mg/mL of USP Cefotetan RS in methanol, acetonitrile, and water (5:5:90), prepared as follows. Place a suitable quantity of USP Cefotetan RS in a suitable volumetric flask, and add methanol, using 5% of the final volume. Swirl for several min, and add acetonitrile, using 5% of the final volume. Swirl until dissolved, and dilute with water to volume.  
**System suitability solution:** Transfer 10 mL of *Standard solution* to a glass-stoppered flask containing a few mg of magnesium carbonate. Sonicate for 10 min. If the solution is not turbid, add a few more mg of magnesium carbonate, and repeat the sonication. Pass the turbid solution through a filter of 0.5-μm or finer pore size. Use the clear filtrate.  
**Sample solution:** 0.2 mg/mL of Cefotetan in methanol, acetonitrile, and water (5:5:90), prepared as follows. Place a suitable quantity of Cefotetan in a suitable volumetric flask, and add methanol, using 5% of the final volume. Swirl for several min, and add acetonitrile, using 5% of the final volume. Swirl until dissolved, and dilute with water to volume.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

**System suitability**Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for cefotetan and cefotetan tautomer are 0.75 and 1.0, respectively.]

**Suitability requirements**Resolution: NLT 2.0 between cefotetan and cefotetan tautomer, *System suitability solution*Column efficiency: NLT 1500 theoretical plates, *Standard solution*Tailing factor: NMT 1.5, *Standard solution*Relative standard deviation: NMT 2.0%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of cefotetan

(C<sub>17</sub>H<sub>17</sub>N<sub>7</sub>O<sub>8</sub>S<sub>4</sub>) in the portion of Cefotetan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Cefotetan RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Cefotetan in the *Sample solution* (mg/mL) $P$  = potency of cefotetan in USP Cefotetan RS (µg/mg)

Acceptance criteria: 950–1030 µg/mg on the anhydrous basis

**SPECIFIC TESTS**

- **STERILITY TESTS** (71): Where the label states that Cefotetan is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*, except use *Fluid A* to each 1000 mL of which has been added 10 g of sodium bicarbonate before sterilization.
- **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that Cefotetan is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.17 USP Endotoxin Unit/mg of cefotetan.
- **WATER DETERMINATION, Method I** (921): NMT 2.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** (11)
  - USP Cefotetan RS
  - USP Endotoxin RS

**Cefotetan Injection****DEFINITION**

Cefotetan Injection is a sterile isoosmotic solution of Cefotetan and Sodium Bicarbonate in Water for Injection. It contains one or more buffer substances and a tonicity-adjusting agent. It contains NLT 90.0% and NMT 120.0% of the labeled amount of cefotetan (C<sub>17</sub>H<sub>17</sub>N<sub>7</sub>O<sub>8</sub>S<sub>4</sub>).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE**

Protect the *Standard solution*, the *System suitability solution*, and the *Sample solution* from light. Use the *Standard solution* and the *System suitability solution* within 2 h. Use the *Sample solution* within 10 min.

**Mobile phase:** Methanol, acetonitrile, glacial acetic acid, and 0.1 M phosphoric acid (105:105:100:1700)

**Standard solution:** 0.2 mg/mL of USP Cefotetan RS in methanol, acetonitrile, and water (5:5:90), prepared as follows. Place a suitable quantity of USP Cefotetan RS in a suitable volumetric flask, and add methanol, using 5% of the final volume. Swirl for several min, and add acetonitrile, using 5% of the final volume. Swirl until dissolved, and dilute with water to volume.

**System suitability solution:** Transfer 10 mL of *Standard solution* to a glass-stoppered flask containing a few mg of magnesium carbonate. Sonicate for 10 min. If the solution is not turbid, add a few more mg of magnesium carbonate, and repeat the sonication. Pass the turbid solution through a filter of 0.5-µm or finer pore size. Use the clear filtrate.

**Sample solution:** 0.2 mg/mL of cefotetan in methanol, acetonitrile, and water (5:5:90) from Injection, prepared as follows. Allow the contents of a container of Injection to thaw, and mix the resultant solution. Transfer a suitable aliquot of this solution to a suitable volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

**System suitability**Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cefotetan and cefotetan tautomer are 0.75 and 1.0, respectively.]

**Suitability requirements**Resolution: NLT 2.0 between cefotetan and cefotetan tautomer, *System suitability solution*Column efficiency: NLT 1500 theoretical plates, *Standard solution*Tailing factor: NMT 1.5, *Standard solution*Relative standard deviation: NMT 2.0%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefotetan (C<sub>17</sub>H<sub>17</sub>N<sub>7</sub>O<sub>8</sub>S<sub>4</sub>) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Cefotetan RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of cefotetan in the *Sample solution* (mg/mL) $P$  = potency of cefotetan in USP Cefotetan RS (µg/mg) $F$  = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–120.0%

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.17 USP Endotoxin Unit/mg of cefotetan



- **STERILITY TESTS** (71): Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **PH** (791): 4.0–6.5
- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.
- **LABELING:** Meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just before use, describes the conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- **USP REFERENCE STANDARDS** (11)  
USP Cefotetan RS  
USP Endotoxin RS

## Cefotetan for Injection

### DEFINITION

Cefotetan for Injection contains an amount of Cefotetan Disodium equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of cefotetan ( $C_{17}H_{17}N_7O_8S_4$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the appropriate *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Sodium** (191): Meets the requirements

### ASSAY

#### • PROCEDURE

[NOTE—Protect the *Standard solution*, the *System suitability solution*, *Sample solution A*, and *Sample solution B* from light, and use within 2 h.]

**Solution A:** Acetonitrile, methanol, and water (1:1:18)

**Mobile phase:** Acetonitrile, methanol, glacial acetic acid, and 0.1 M phosphoric acid (105:105:100:1700)

**Standard solution:** 20 mg of USP Cefotetan RS in a 100-mL volumetric flask. Add 5 mL of methanol, swirl for several min, add 5 mL of acetonitrile, and swirl until dissolved. Dilute with water to volume.

**System suitability solution:** 10 mL of *Standard solution* in a glass-stoppered flask containing a few mg of magnesium carbonate. Sonicate for 10 min. If the solution is not turbid, add a few more mg of magnesium carbonate, and repeat the sonication. Filter the turbid solution through a filter of 0.5- $\mu$ m or finer pore size. Use the clear filtrate.

**Sample solution A** (where the package is represented as being in a single-dose container): Constitute Cefotetan for Injection as directed in the labeling. Withdraw all of the withdrawable contents, and quantitatively dilute with *Solution A* to obtain a solution containing the equivalent of 200  $\mu$ g/mL of cefotetan.

**Sample solution B** (where the label states the quantity of cefotetan in a given volume of constituted solution): Constitute Cefotetan for Injection as directed in the labeling. Dilute an aliquot of the constituted solution with *Solution A* to obtain a solution containing the equivalent of 200  $\mu$ g/mL of cefotetan.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection size:** 20  $\mu$ L

### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for cefotetan and cefotetan tautomer are 0.75 and 1.0, respectively, *System suitability solution*.]

### Suitability requirements

**Resolution:** NLT 2.0 between cefotetan and cefotetan tautomer, *System suitability solution*

**Column efficiency:** NLT 1500 theoretical plates, *Standard solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution*, and *Sample solution A* or *Sample solution B*

Calculate the percentage of  $C_{17}H_{17}N_7O_8S_4$  withdrawn from the container, or in the portion of solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from *Sample solution A* or *Sample solution B*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cefotetan RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of cefotetan in *Sample solution A* or *Sample solution B* ( $\mu$ g/mL)

**Acceptance criteria:** 90.0%–120.0%

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

### SPECIFIC TESTS

- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** (1), *Specific Tests, Completeness and clarity of solutions*: Meets the requirements at the time of use
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.17 USP Endotoxin Unit/mg of cefotetan
- **STERILITY TESTS** (71): Meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections
- **PH** (791): 4.0–6.5, in a solution 100 mg/mL
- **WATER DETERMINATION, Method Ic** (921): NMT 2.8%
- **OTHER REQUIREMENTS:** It meets the requirements under *Labeling* (7), *Labels and Labeling for Injectable Products*.

### ADDITIONAL REQUIREMENTS

#### Change to read:

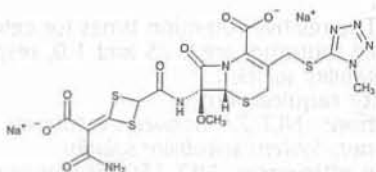
- **PACKAGING AND STORAGE:** Preserve as described under *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).



• **USP REFERENCE STANDARDS (11)**

USP Cefotetan RS  
USP Endotoxin RS

## Cefotetan Disodium



$C_{17}H_{15}N_7Na_2O_8S_4$  619.58

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[4-(2-amino-1-carboxy-2-oxoethylidene)-1,3-dithietan-2-yl]carbonyl]amino]-7-methoxy-3-[[[1-methyl-1H-tetrazol-5-yl]thio]methyl]-8-oxo-, disodium salt, [(6R-(6 $\alpha$ ,7 $\alpha$ ))-]; (6R,7S)-4-[[2-Carboxy-7-methoxy-3-[[[1-methyl-1H-tetrazol-5-yl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-7-yl]carbonyl]-1,3-dithietane- $\Delta^{2,\alpha}$ -malonamic acid, disodium salt; (6R,7S)-7-[4-(Carbamoylcarboxymethylene)-1,3-dithietane-2-carboxamido]-7-methoxy-3-[[[1-methyl-1H-tetrazol-5-yl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, disodium salt [74356-00-6].

### DEFINITION

Cefotetan Disodium contains the equivalent of NLT 830  $\mu$ g/mg and NMT 970  $\mu$ g/mg of cefotetan ( $C_{17}H_{15}N_7O_8S_4$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Sodium (191):** Meets the requirements

### ASSAY

#### • PROCEDURE

Protect the *Standard solution*, the *System suitability solution*, and the *Sample solution* from light, and use within 2 h.

**Mobile phase:** Methanol, acetonitrile, glacial acetic acid, and 0.1 M phosphoric acid (105:105:100:1700)

**Standard solution:** 0.2 mg/mL of USP Cefotetan RS in methanol, acetonitrile, and water (5:5:90), prepared as follows. Place a suitable quantity of USP Cefotetan RS in a suitable volumetric flask, and add methanol, using 5% of the final volume. Swirl for several min, and add acetonitrile, using 5% of the final volume. Swirl until dissolved, and dilute with water to volume.

**System suitability solution:** Transfer 10 mL of *Standard solution* to a glass-stoppered flask containing a few mg of magnesium carbonate. Sonicate for 10 min. If the solution is not turbid, add a few more mg of magnesium carbonate, and repeat the sonication. Pass the turbid solution through a filter of 0.5- $\mu$ m or finer pore size. Use the clear filtrate.

**Sample solution:** 0.2 mg/mL of Cefotetan Disodium in methanol, acetonitrile, and water (5:5:90), prepared as follows. Place a suitable quantity of Cefotetan Disodium in a suitable volumetric flask, and add methanol, using 5% of the final volume. Swirl for several min, and add acetonitrile, using 5% of the final volume. Swirl until dissolved, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for cefotetan and cefotetan tautomer are 0.75 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between cefotetan and cefotetan tautomer, *System suitability solution*

**Column efficiency:** NLT 1500 theoretical plates, *Standard solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in  $\mu$ g/mg, of cefotetan ( $C_{17}H_{15}N_7O_8S_4$ ) in the portion of Cefotetan Disodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cefotetan RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cefotetan Disodium in the *Sample solution* (mg/mL)

$P$  = potency of cefotetan in USP Cefotetan RS ( $\mu$ g/mg)

**Acceptance criteria:** 830–970  $\mu$ g/mg on the anhydrous basis

### SPECIFIC TESTS

#### • pH (791)

**Sample solution:** 100 mg/mL

**Acceptance criteria:** 4.0–6.5

#### • WATER DETERMINATION, Method 1c (921):

NMT 2.5%

#### • STERILITY TESTS (71):

Where the label states that Cefotetan Disodium is sterile, it meets the requirements

when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

#### • BACTERIAL ENDOTOXINS TEST (85):

Where the label states that Cefotetan Disodium is sterile or it must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.17 USP Endotoxin Unit/mg of cefotetan.

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE:

Preserve in tight containers.

#### • LABELING:

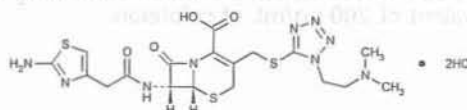
Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

#### • USP REFERENCE STANDARDS (11)

USP Cefotetan RS

USP Endotoxin RS

## Cefotiam Hydrochloride



$C_{18}H_{23}N_9O_4S_3 \cdot 2HCl$  598.55



5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(2-amino-4-thiazolyl)acetyl]-amino-3-[[[1-[2-(dimethylamino)ethyl]-1H-tetrazol-5-yl]-thio]methyl]-8-oxo, hydrochloride, (6R-trans)-  
 (6R,7R)-7-[2-(2-Amino-4-thiazolyl)acetamido]-3-[[[1-[2-(dimethylamino)ethyl]-1H-tetrazol-5-yl]thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrochloride.  
 7(R)-[2-(2-Amino-4-thiazolyl)acetamido]-3-[[[1-[2-(dimethylamino)ethyl]-1H-tetrazol-5-yl]thio]methyl]-3-cephem-4-carboxylic acid dihydrochloride [66309-69-1].

» Cefotiam Hydrochloride contains the equivalent of not less than 790 µg and not more than 925 µg of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards (11)**—  
 USP Cefotiam Hydrochloride RS

**Identification**—

**A: Ultraviolet Absorption (197U)**—

**Solution:** 20 µg per mL.

**Medium:** water.

**B:** The retention time of the cefotiam peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Crystallinity (695):** meets the requirements.

**Pyrogen**—Where the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements of the *Pyrogen Test* (151), the test dose being 1.0 mL per kg of a solution in pyrogen-free sodium carbonate solution (prepared by dissolving 25.6 g of sodium carbonate, previously heated at 170° for not less than 4 hours, in 1000 mL of Sterile Water for Injection) containing 40 mg per mL.

**Sterility Tests (71)**—Where the label states that it is sterile, it meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**Water Determination, Method I (921):** not more than 7.0%, the *Test Preparation* being prepared as directed for a hygroscopic specimen, except to use a mixture of 20 mL of formamide (previously dried over anhydrous sodium sulfate for 24 hours) and methanol (2:1), instead of methanol, to dissolve the specimen, and to determine the water content of the formamide and methanol mixture.

**Assay**—

**Mobile phase**—Dissolve 13.1 g of ammonium sulfate in 850 mL of water, adjust with 2 N ammonium hydroxide to a pH of  $6.5 \pm 0.1$ , add 150 mL of acetonitrile, and mix. Filter through a suitable filter of 0.5 µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability under Chromatography (621)*).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cefotiam Hydrochloride RS, quantitatively in water to obtain a solution having a known concentration of about 1000 µg of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 50 µg of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) per mL. Use this solution without delay.

**Assay preparation**—Transfer about 60 mg of Cefotiam Hydrochloride, accurately weighed, to a 50-mL volumetric

flask, add water to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Use this solution without delay.

**System suitability solution**—Prepare a solution of USP Cefotiam Hydrochloride RS in water containing about 1 mg per mL. Heat this solution at 95° for 3 minutes, and cool. Transfer 1 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography (621)*)—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency, determined from the cefotiam peak, is not less than 1985 theoretical plates when calculated by the formula:

$$5.545(t_r / W_{H/2})^2$$

the tailing factor for the cefotiam peak is not more than 1.8, and the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.6 for de-tetrazol-cefotiam and 1.0 for cefotiam; and the resolution,  $R$ , between the de-tetrazol-cefotiam peak and the cefotiam peak is not less than 4.0.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) in each mg of the Cefotiam Hydrochloride taken by the formula:

$$1000(C/W)(r_u / r_s)$$

in which  $C$  is the concentration, in µg per mL, of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) in the *Standard preparation*, based on the quantity of USP Cefotiam Hydrochloride RS taken to prepare the *Standard preparation*, the designated cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) content, in µg per mg, of USP Cefotiam Hydrochloride RS, and the extent of dilution;  $W$  is the weight, in mg, of Cefotiam Hydrochloride taken to prepare the *Assay preparation*; and  $r_u$  and  $r_s$  are the cefotiam peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefotiam for Injection

» Cefotiam for Injection contains an amount of Cefotiam Hydrochloride equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ). It may contain Sodium Carbonate.

**Change to read:**

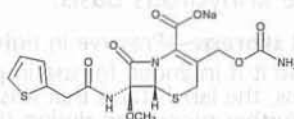
**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements (659)*, *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

**USP Reference standards (11)**—  
 USP Cefotiam Hydrochloride RS



**Identification—****A: Ultraviolet Absorption (197U)—****Solution:** 20 µg per mL.**Medium:** water.**B:** The retention time of the cefotiam peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*; as obtained in the *Assay*.**Pyrogen—**It meets the requirements of the *Pyrogen Test* (151), the test dose being 1.0 mL per kg of a solution prepared by diluting Cefotiam for Injection with Sterile Water for Injection to a concentration of 40 mg of cefotiam per mL.**Sterility Tests (71)—**It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.**pH (791):** between 5.7 and 7.2, in a solution containing the equivalent of 100 mg of cefotiam per mL.**Loss on drying (731)—**Dry about 100 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 6.0% of its weight.**Particulate Matter in Injections (788):** meets the requirements for small-volume injections.**Assay—***Mobile phase, Standard preparation, System suitability solution, and Chromatographic system—*Prepare as directed in the *Assay under Cefotiam Hydrochloride*.*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute a container of Cefotiam for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing the equivalent of about 1 mg of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 50 µg of cefotiam per mL. Use this solution without delay.*Assay preparation 2* (where the label states the quantity of cefotiam in a given volume of constituted solution)—Constitute a container of Cefotiam for Injection in a volume of water, accurately measured, equivalent to the volume of diluent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with water to obtain a solution containing about 1 mg of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) per mL. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains the equivalent of about 50 µg of cefotiam per mL. Use this solution without delay.*Procedure—*Proceed as directed for *Procedure* in the *Assay under Cefotiam Hydrochloride*. Calculate the quantity, in mg, of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$C(L/D)(r_U/r_S)$$

in which *C* is the concentration, in µg per mL, of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) in the *Standard preparation*, based on the quantity of USP Cefotiam Hydrochloride RS taken to prepare the *Standard preparation*, the designated cefotiam(C<sub>18</sub>H<sub>23</sub>N<sub>9</sub>O<sub>4</sub>S<sub>3</sub>) content, in µg per mg, of USP Cefotiam Hydrochloride RS, and the extent of dilution; *L* is the labeled quantity, in mg, of cefotiam ( $C_{18}H_{23}N_9O_4S_3$ ) in the container, or in the volume of constituted solution taken; *D* is the concentration, in µg of cefotiam per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the volume of constituted solution taken, respectively, and the extent of dilution; and *r<sub>U</sub>* and *r<sub>S</sub>* are the cefotiam peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.**Cefoxitin Sodium** $C_{16}H_{16}N_3NaO_7S_2$  449.435-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[[(aminocarbonyl)oxy]methyl]-7-methoxy-8-oxo-7-[(2-thienylacetyl)amino]-], sodium salt (6*R*-*cis*-); Sodium (6*R*,7*S*)-3-(hydroxymethyl)-7-methoxy-8-oxo-7-[2-(2-thienyl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate carbamate (ester) [33564-30-6].**DEFINITION**Cefoxitin Sodium contains the equivalent of NLT 927 µg/mg and NMT 970 µg/mg of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ), corresponding to NLT 97.5% and NMT 102.0% of cefoxitin sodium ( $C_{16}H_{16}N_3NaO_7S_2$ ), calculated on the anhydrous and acetone- and methanol-free basis.**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. ULTRAVIOLET ABSORPTION (197U)**  
**Buffer:** 1.0 g/L of monobasic potassium phosphate and 1.8 g/L of anhydrous dibasic sodium phosphate in water  
**Sample solution:** 20 µg/mL in *Buffer*  
**Acceptance criteria:** Meets the requirements
- **C. IDENTIFICATION TESTS—GENERAL, Sodium (191)**  
**Sample solution:** 50 mg/mL  
**Acceptance criteria:** Meets the requirements

**ASSAY****• PROCEDURE****Buffer:** Dissolve 1.0 g of monobasic potassium phosphate and 1.8 g of dibasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of 7.1 ± 0.1, and dilute with water to 1 L. Pass through a membrane filter of 1-µm or finer pore size.**Mobile phase:** Acetonitrile, water, and glacial acetic acid (160:840:10). Pass through a membrane filter of 1-µm or finer pore size.**Standard solution:** 0.3 mg/mL of USP Cefoxitin RS in *Buffer*. Sonicate, if necessary, to dissolve. Use this solution within 5 h.**Sample solution:** 0.3 mg/mL of Cefoxitin Sodium in *Buffer*. Use this solution within 5 h.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 5- to 10-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

**System suitability**Sample: *Standard solution***Suitability requirements**

Column efficiency: NLT 2800 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the quantity, in μg/mg, of cefoxitin (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>7</sub>S<sub>2</sub>) in the portion of Cefoxitin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of the *Standard solution* (mg/mL) $C_U$  = concentration of the *Sample solution* (mg/mL) $P$  = potency of cefoxitin in USP Cefoxitin RS (mg/mg) $F$  = conversion factor, 1000 μg/mg

Acceptance criteria: 927–970 μg/mg of cefoxitin, corresponding to NLT 97.5% and NMT 102.0% of cefoxitin sodium, on the anhydrous and acetone- and methanol-free basis

**IMPURITIES****Delete the following:**

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm (Official 1-Jan-2018)

• **LIMIT OF ACETONE AND METHANOL**

Standard stock solution 1: 0.5% v/v of acetone in water

Standard stock solution 2: 0.5% v/v of methanol in water

Standard solution: 0.050% v/v of acetone from *Standard stock solution 1* and 0.005% v/v of methanol from *Standard stock solution 2* in water

Sample solution: Transfer 5.0 g of Cefoxitin Sodium to a 50-mL volumetric flask, and dissolve in and dilute with water to volume. Transfer 3.0 mL of the resulting solution to a 15-mL centrifuge tube, cool in an ice-water bath for 2 min, and add 3.0 mL of 0.24 N hydrochloric acid while swirling vigorously. Centrifuge to obtain a clear solution.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

**Columns**

Precolumn: Packed with 60- to 80-mesh silane-treated glass beads

Analytical: 6.3-mm × 1.8-m glass; support S2

**Temperatures**

Columns: 110°

Injection port: 100°

Detector: 200°

Carrier gas: Nitrogen

Flow rate: 50 mL/min

Injection volume: 2 μL

**System suitability**Sample: *Standard solution***Suitability requirements**

Column efficiency: NLT 160 theoretical plates for acetone and NLT 200 theoretical plates for methanol

Tailing factor: NMT 1.3 for acetone and NMT 2.3 for methanol

Relative standard deviation: NMT 5%

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentages of acetone and methanol in the portion of Cefoxitin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times D$$

 $r_U$  = peak area of acetone or methanol in the *Sample solution* $r_S$  = peak area of acetone or methanol in the *Standard solution* $C_S$  = concentration of acetone or methanol in the *Standard solution* (% v/v) $C_U$  = concentration of Cefoxitin Sodium in the *Sample solution* (g/mL) $D$  = density of acetone or methanol at 20° (g/mL)

Acceptance criteria: NMT 0.7% of acetone and NMT 0.1% of methanol

**SPECIFIC TESTS**• **OPTICAL ROTATION**, *Specific Rotation* (781S)

Sample solution: 10 mg/mL in methanol

Acceptance criteria: +206° to +214°, calculated on the anhydrous and acetone- and methanol-free basis

• **CRYSTALLINITY** (695): Meets the requirements• **pH** (791)

Sample solution: 100 mg/mL

Acceptance criteria: 4.2–7.0

• **WATER DETERMINATION**, *Method I* (921)

Analysis: Use a mixture of ethylene glycol and pyridine (3:1) in the titration vessel in place of methanol.

Acceptance criteria: NMT 1.0%

• **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that Cefoxitin Sodium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.13 USP Endotoxin Unit/mg of cefoxitin.• **STERILITY TESTS** (71): Where the label states that Cefoxitin Sodium is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE**: Preserve in tight containers, and store in a cold place.• **LABELING**: Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.



• **USP REFERENCE STANDARDS** (11)

USP Cefoxitin RS  
USP Endotoxin RS

## Cefoxitin Injection

### DEFINITION

Cefoxitin Injection is a sterile solution of Cefoxitin Sodium and one or more suitable buffer substances in Water for Injection. It contains Dextrose or Sodium Chloride as a tonicity-adjusting agent. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** Dissolve 1.0 g of monobasic potassium phosphate and 1.8 g of dibasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of  $7.1 \pm 0.1$ , and dilute with water to 1 L. Pass through a membrane filter of 1- $\mu$ m or finer pore size.

**Mobile phase:** Acetonitrile, water, and glacial acetic acid (160:840:10). Pass through a membrane filter of 1- $\mu$ m or finer pore size.

**Standard solution:** 0.3 mg/mL of USP Cefoxitin RS in *Buffer*. Sonicate, if necessary, to dissolve. Use this solution within 5 h.

**Sample solution:** 0.3 mg/mL of cefoxitin prepared as follows. Allow one container of Injection to thaw, and mix. Dilute an aliquot of Injection with *Buffer* to a suitable volume. Use this solution within 5 h.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; 5- to 10- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 2800 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 1.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefoxitin in the *Sample solution* (mg/mL)

$P$  = potency of cefoxitin in USP Cefoxitin RS (mg/mg)

Acceptance criteria: 90.0%–120.0%

### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.13 USP Endotoxin Unit/mg of cefoxitin
- **STERILITY TESTS** (71): It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.
- **pH** (791): 4.5–8.0
- **PARTICULATE MATTER IN INJECTIONS** (788): It meets the requirements for small-volume injections.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.
- **LABELING:** It meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just before use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- **USP REFERENCE STANDARDS** (11)  
USP Cefoxitin RS  
USP Endotoxin RS

## Cefoxitin for Injection

### DEFINITION

Cefoxitin for Injection contains Cefoxitin Sodium equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ).

### ASSAY

#### • PROCEDURE

**Buffer:** Dissolve 1.0 g of monobasic potassium phosphate and 1.8 g of dibasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of  $7.1 \pm 0.1$ , and dilute with water to 1 L. Pass through a membrane filter of 1- $\mu$ m or finer pore size.

**Mobile phase:** Acetonitrile, water, and glacial acetic acid (160:840:10). Pass through a membrane filter of 1- $\mu$ m or finer pore size.

**Standard solution:** 0.3 mg/mL of USP Cefoxitin RS in *Buffer*. Sonicate, if necessary, to dissolve. Use this solution within 5 h.

**Sample solution 1** (where it is represented as being in a single-dose container): Nominally 0.3 mg/mL of cefoxitin prepared as follows. Constitute Cefoxitin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, transfer to a suitable volumetric flask, and dilute with water to volume. Use this solution within 5 h.

**Sample solution 2** (where the label states the quantity of cefoxitin in a given volume of constituted solution): Nominally 0.3 mg/mL of cefoxitin prepared as follows. Constitute Cefoxitin for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Transfer a suitable aliquot of the constituted solution to a suitable volumetric flask, and dilute with water to volume. Use this solution within 5 h.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)



Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; 5- to 10-μm packing L1

Flow rate: 1 mL/min

Injection volume: 10 μL

System suitability

Sample: Standard solution

Suitability requirements

Column efficiency: NLT 2800 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

Analysis

Samples: Sample solution 1 or Sample solution 2 and Standard solution

Calculate the percentage of the labeled amount of cefoxitin ( $C_{16}H_{17}N_3O_7S_2$ ) in the portion of Cefoxitin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of the Standard solution (mg/mL)

$C_U$  = nominal concentration of cefoxitin in Sample solution 1 or Sample solution 2 (mg/mL)

$P$  = potency of cefoxitin in USP Cefoxitin RS (mg/mg)

Acceptance criteria: 90.0%–120.0%

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

#### SPECIFIC TESTS

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.13 USP Endotoxin Unit/mg of cefoxitin
- **STERILITY TESTS (71):** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **PARTICULATE MATTER IN INJECTIONS (788):** It meets the requirements for small-volume injections.
- **pH (791)**  
Sample solution: 100 mg/mL  
Acceptance criteria: 4.2–7.0
- **WATER DETERMINATION, Method I (921)**  
Analysis: Use a mixture of ethylene glycol and pyridine (3:1) in the titration vessel in place of methanol.  
Acceptance criteria: NMT 1.0%
- **OTHER REQUIREMENTS:** It meets the requirements of the *Identification tests in Cefoxitin Sodium*. It meets the requirements in *Labeling (7)*, *Labels and Labeling for Injectable Products*.

#### ADDITIONAL REQUIREMENTS

##### Change to read:

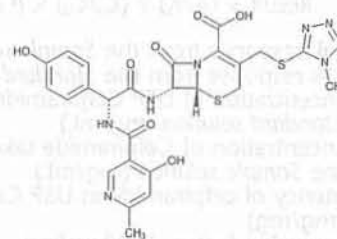
- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements (659)*, *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

#### • USP REFERENCE STANDARDS (11)

USP Cefoxitin RS

USP Endotoxin RS

### Cefpiramide



$C_{25}H_{24}N_8O_7S_2$  612.64

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(4-hydroxy-6-methyl-3-pyridinyl)carbonyl]amino]-(4-hydroxyphenyl)acetyl]amino]-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-, [6R-[6α,7β(R\*)]]-; (6R,7R)-7-[(R)-2-(4-Hydroxy-6-methylnicotinamido)-2-(p-hydroxyphenyl)acetamido]-3-[[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [70797-11-4].

#### DEFINITION

Cefpiramide contains NLT 974 μg/mg and NMT 1026 μg/mg of  $C_{25}H_{24}N_8O_7S_2$ , calculated on the anhydrous basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Buffer:** 1.36 g/L of monobasic potassium phosphate in water adjusted with 1 N sodium hydroxide to a pH of  $6.8 \pm 0.1$  prior to final dilution

**Mobile phase:** Tetrahydrofuran, acetonitrile, methanol, and Buffer (40:40:40:880)

**System suitability solution:** 1 mg/mL of USP Cefpiramide RS in 0.01 N sodium hydroxide. Heat this solution at 95° for 10 min. Mix 1 mL of this solution with 19 mL of Mobile phase. This solution contains a mixture of cefpiramide and cefpiramide lactone.

**Standard solution:** 0.25 mg/mL of USP Cefpiramide RS in Mobile phase

**Sample solution:** 0.25 mg/mL of Cefpiramide in Mobile phase

**Chromatographic system**

(See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.0-mm × 15- to 30-cm; 5- to 10-μm packing L7

Flow rate: 1.5 mL/min

Injection size: 20 μL

System suitability

Samples: System suitability solution and Standard solution

[NOTE—The relative retention times for cefpiramide and cefpiramide lactone are about 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 5 between cefpiramide lactone and cefpiramide, System suitability solution



Tailing factor: 0.95–1.4, *Standard solution*  
Relative standard deviation: NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the quantity, in  $\mu\text{g}$ , of cefpiramide ( $\text{C}_{25}\text{H}_{24}\text{N}_8\text{O}_7\text{S}_2$ ) in each mg of the portion of Cefpiramide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Cefpiramide RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Cefpiramide taken to prepare the *Sample solution* (mg/mL)  
 $P$  = potency of cefpiramide in USP Cefpiramide RS (mg/mg)  
 $F$  = conversion factor, 1000  $\mu\text{g}/\text{mg}$   
Acceptance criteria: 974–1026  $\mu\text{g}/\text{mg}$  on the anhydrous basis

**IMPURITIES****• ORGANIC IMPURITIES**

**System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Buffer:** 4.08 g/L of monobasic potassium phosphate in water, adjusted with 1 N sodium hydroxide to a pH of  $7.5 \pm 0.1$  prior to final dilution

**Mobile phase:** Methanol and *Buffer* (250:750)

**Standard stock solution:** 0.15 mg/mL of sodium 5-mercapto-1-methyltetrazole and 0.25 mg/mL of USP Cefpiramide RS in *Buffer*

**Standard solution:** 3  $\mu\text{g}/\text{mL}$  of sodium 5-mercapto-1-methyltetrazole and 5  $\mu\text{g}/\text{mL}$  of USP Cefpiramide RS from the *Standard stock solution* in *Mobile phase*

**Sample solution:** 0.5 mg/mL of Cefpiramide in *Mobile phase*

**N-Ethylmaleimide solution:** 40 mg/mL of N-ethylmaleimide in methanol

**Test preparation:** 10 mg/mL of sodium 5-mercapto-1-methyltetrazole in *N-Ethylmaleimide solution* in a stoppered centrifuge tube. Sonicate for 15 min.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Determine the water content of sodium 5-mercapto-1-methyltetrazole by the titrimetric method (see *Water Determination* (921)), using 5.0 mL of the *Test preparation*.

Calculate the percentage of 5-mercapto-1-methyltetrazole in the portion of Cefpiramide taken:

$$\text{Result} = (r_U/r_S) \times (M_{r1}/M_{r2}) \times (C_S/C_U) \times F \times 100$$

- $r_U$  = peak response of 5-mercapto-1-methyltetrazole from the *Sample solution*  
 $r_S$  = peak response of 5-mercapto-1-methyltetrazole from the *Standard solution*  
 $M_{r1}$  = molecular weight of 5-mercapto-1-methyltetrazole, 115.14  
 $M_{r2}$  = molecular weight of anhydrous sodium 5-mercapto-1-methyltetrazole, 138.13  
 $C_S$  = concentration of sodium 5-mercapto-1-methyltetrazole, corrected for water, in the *Standard solution* ( $\mu\text{g}/\text{mL}$ )  
 $C_U$  = concentration of Cefpiramide in the *Sample solution* (mg/mL)  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
Calculate the percentage of each other impurity in the portion of Cefpiramide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

- $r_U$  = peak response of each other impurity from the *Sample solution*  
 $r_S$  = peak response of cefpiramide from the *Standard solution*  
 $C_S$  = concentration of USP Cefpiramide RS in the *Standard solution* ( $\mu\text{g}/\text{mL}$ )  
 $C_U$  = concentration of Cefpiramide in the *Sample solution* ( $\mu\text{g}/\text{mL}$ )  
 $P$  = potency of cefpiramide in USP Cefpiramide RS ( $\mu\text{g}/\text{mg}$ )

Acceptance criteria: See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
5-Mercapto-1-methyltetrazole	0.20	0.7
Cefpiramide	1.0	—
Any other individual impurity	—	0.7
Total impurities	—	2.0

**SPECIFIC TESTS****• OPTICAL ROTATION, Specific Rotation (781S)**

**Sample solution:** 10 mg/mL, in dimethylformamide  
Acceptance criteria:  $-100^\circ$  to  $-112^\circ$

**• CRYSTALLINITY (69S):** Meets the requirements**• PH (791)**

**Sample solution:** 5-mg/mL suspension in water  
Acceptance criteria: 3.0–5.0

**• WATER DETERMINATION, Method I (921):** NMT 9.0%**• PYROGEN TEST (151)**

**Sample solution:** 50 mg/mL of cefpiramide in Sterile Water for Injection

**Test dose:** 1.0 mL/kg of the *Sample solution*

Acceptance criteria: Where the label states that Cefpiramide is sterile, or it must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements.

**• STERILITY TESTS (71):** Where the label states that Cefpiramide is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers.
- LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- USP REFERENCE STANDARDS (11)**  
USP Cefpiramide RS

**Cefpiramide for Injection****DEFINITION**

Cefpiramide for Injection contains NLT 90.0% and NMT 120.0% of the labeled amount of cefpiramide ( $\text{C}_{25}\text{H}_{24}\text{N}_8\text{O}_7\text{S}_2$ ).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Buffer:** 1.36 g/L of monobasic potassium phosphate in water adjusted with 1 N sodium hydroxide to a pH of  $6.8 \pm 0.1$  before final dilution



**Mobile phase:** Tetrahydrofuran, acetonitrile, methanol, and Buffer (40:40:40:880)

**System suitability solution:** 1 mg/mL of USP Cefpiramide RS in 0.01 N sodium hydroxide. Heat this solution at 95° for 10 min. Dilute 1 mL of this solution with *Mobile phase* to 20 mL. This solution contains a mixture of cefpiramide and cefpiramide lactone.

**Standard solution:** 0.25 mg/mL of USP Cefpiramide RS in *Mobile phase*

**Sample solution 1** (where it is represented as being in a single-dose container): Constitute a container of Cefpiramide for Injection in a volume of water corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute with *Mobile phase* to obtain a solution containing the nominal equivalent of 0.25 mg/mL of cefpiramide.

**Sample solution 2** (where the label states the quantity of cefpiramide in a given volume of constituted solution): Constitute a container of Cefpiramide for Injection in a volume of water equivalent to the volume of diluent specified in the labeling. Dilute the constituted solution with water to obtain a solution nominally containing 0.25 mg/mL of cefpiramide.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.0-mm × 15- to 30-cm; 5- to 10-μm packing L7

**Flow rate:** 1.5 mL/min

**Injection size:** 20 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cefpiramide and cefpiramide lactone are 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 5 between cefpiramide lactone and cefpiramide, *System suitability solution*

**Tailing factor:** 0.95–1.4, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of cefpiramide ( $C_{25}H_{24}N_8O_7S_2$ ) withdrawn from the container, or in the portion of constituted solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the *Sample solution*

$r_S$  = peak response of the *Standard solution*

$C_S$  = concentration of USP Cefpiramide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cefpiramide in *Sample solution 1* or *Sample solution 2* (mg/mL)

**Acceptance criteria:** 90.0%–120.0%

#### SPECIFIC TESTS

##### • PYROGEN TEST (151)

**Sample solution:** 50 mg/mL of cefpiramide from Cefpiramide for Injection in Sterile Water for Injection

**Test dose:** 1.0 mL/kg of the *Sample solution*

**Acceptance criteria:** Meets the requirements

##### • STERILITY TESTS (71): It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.

##### • PH (791)

**Sample solution:** Equivalent to 100 mg/mL of cefpiramide from Cefpiramide for Injection

**Acceptance criteria:** 6.0–8.0 in water

##### • WATER DETERMINATION, Method I (921): NMT 3.0%

##### • PARTICULATE MATTER IN INJECTIONS (788): It meets the requirements for small-volume injections.

#### ADDITIONAL REQUIREMENTS

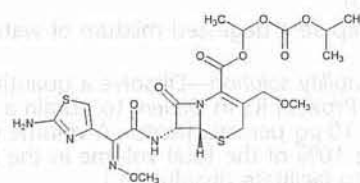
##### Change to read:

##### • PACKAGING AND STORAGE: Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

##### • USP REFERENCE STANDARDS (11)

USP Cefpiramide RS

## Cefpodoxime Proxetil



$C_{21}H_{27}N_5O_9S_2$  557.60

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-carboxylic acid, 7-[[[(2-amino-4-thiazolyl)(methoxymimino)acetyl] amino]-3-(methoxymethyl)-8-oxo-1-[[[(1-methylethoxy) carbonyl]oxy]ethyl ester, [6R-[6α,7β(Z)]]- (±)-1-Hydroxyethyl(+)-(6R,7R)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-3-methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, 7<sup>2</sup>-(Z)-(O-methyloxime), isopropyl carbonate (ester) [87239-81-4].

» Cefpodoxime Proxetil contains the equivalent of not less than 690 μg and not more than 804 μg of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers, at a temperature not exceeding 25°.

#### USP Reference standards (11)—

USP Cefpodoxime Proxetil RS

#### Identification—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 15 μg per mL.

*Medium:* acetonitrile.

**C:** Dissolve 1 mg of it in 4 mL of water, add 1 mL of 1 N sulfuric acid while cooling in an ice bath, add 1 mL of a freshly prepared solution of sodium nitrite (1 in 100), allow to stand for 2 minutes, then add 1 mL of ammonium sulfamate solution (1 in 100). Allow to stand for 1 minute, and add 1 mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride TS: a red-purple color develops.

**Specific rotation** (781S): between +35.0° and +48.0°.

*Test solution:* 10 mg per mL, in methanol.

**Water Determination, Method I** (921): not more than 3.0%.



**Residue on ignition** (281): not more than 0.2%.

**Delete the following:**

• **Heavy metals, Method II** (231): 0.002%. (Official 1-Jan-2018)

**Isomer ratio**—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, calculate the ratio of the cefpodoxime proxetil *R*-epimer peak response to the sum of the peak responses of the cefpodoxime proxetil *S*-epimer peak and the cefpodoxime proxetil *R*-epimer peak: the ratio is between 0.5 and 0.6.

**Chromatographic purity**—

**Solution A**—Prepare filtered and degassed 0.02 M ammonium acetate.

**Solution B**—Use filtered and degassed acetonitrile.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a degassed mixture of water and acetonitrile (2:1).

**System suitability solution**—Dissolve a quantity of USP Cefpodoxime Proxetil RS in *Diluent* to obtain a solution containing about 10 µg per mL. [NOTE—A volume of methanol not exceeding 10% of the total volume in the final solution may be used to facilitate dissolution.]

**Test solution**—Transfer about 50 mg of Cefpodoxime Proxetil, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, using sonication if necessary, dilute with *Diluent* to volume, and mix. This solution should be injected promptly, but may be analyzed within 24 hours when stored at 8°.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 260-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The column temperature is maintained at a constant temperature of about 30°. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	90	10	equilibration (10 minutes)
0–10	90→68	10→32	linear gradient
10–40	68	32	isocratic
40–80	68→50	32→50	linear gradient
80–85	50	50	isocratic
85–90	50→25	50→75	linear gradient
90–95	25	75	isocratic
95–100	25→90	75→10	linear gradient

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the retention time for cefpodoxime proxetil *R*-epimer is between 37 and 42 minutes; the relative retention times are about 0.9 for cefpodoxime proxetil *S*-epimer and 1.0 for cefpodoxime proxetil *R*-epimer; the resolution, *R*, between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 4.0; the column efficiency is not less than 19,000 theoretical plates determined from the cefpodoxime proxetil *R*-epimer peak; and the relative standard deviation for replicate injections determined from the sum of the areas of the cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer peaks is not more than 2.0%.

**Procedure**—Inject a volume (about 20 µL) of the *Test solution* into the chromatograph, record the chromatogram,

and measure all of the peak areas. Calculate the percentage of each impurity in the portion of Cefpodoxime Proxetil taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak area for each impurity; and  $r_s$  is the sum of the areas of all the peaks: not more than 3.0% of any peak at a relative retention time of about 0.86 is found; not more than 1.0% for any peak at relative retention times of about 1.27, 1.39, and other individual peaks having relative retention times higher than 2.0 is found; not more than 0.5% of any other individual impurity is found; and not more than 6.0% of total impurities is found, impurity peaks of less than 0.05% being disregarded.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of 0.02 M ammonium acetate and acetonitrile (6:4). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a degassed mixture of water and acetonitrile (6:4).

**Standard preparation**—Transfer about 25 mg of USP Cefpodoxime Proxetil RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45-µm or finer porosity.

**Assay preparation**—Transfer about 50 mg of Cefpodoxime Proxetil, accurately weighed, to a 100-mL volumetric flask, dissolve in 10 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45-µm or finer porosity.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 235-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 2 mL per minute. The column temperature is maintained at a constant temperature of about 30°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for cefpodoxime proxetil *S*-epimer and 1.0 for cefpodoxime proxetil *R*-epimer; the resolution, *R*, between cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer is not less than 2.5; the tailing factor for cefpodoxime proxetil *R*-epimer is not more than 1.5; and the relative standard deviation determined from the sum of the areas of the cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer peaks for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity in µg of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in each mg of Cefpodoxime Proxetil taken by the formula:

$$2000(CP/W)(r_u / r_s)$$

in which *C* is the concentration, in mg per mL, of USP Cefpodoxime Proxetil RS in the *Standard preparation*; *P* is the designated potency, in µg per mg, of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in USP Cefpodoxime Proxetil RS; *W* is the weight, in mg, of Cefpodoxime Proxetil taken to prepare the *Assay preparation*; and  $r_u$  and  $r_s$  are the sums of the peak responses for cefpodoxime proxetil *S*-epimer and cefpodoxime proxetil *R*-epimer obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Cefpodoxime Proxetil for Oral Suspension

» Cefpodoxime Proxetil for Oral Suspension contains Cefpodoxime Proxetil and one or more buffers, suspending agents, sweeteners, flavorings, and preservatives. When constituted as directed in the labeling, it contains the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ).

**Packaging and storage**—Preserve in tight containers, at a temperature not exceeding 30°. Store the constituted Oral Suspension in a refrigerator.

**USP Reference standards** (11)—  
USP Cefpodoxime Proxetil RS

**Identification**—The retention times of the cefpodoxime proxetil R-epimer peak and the cefpodoxime proxetil S-epimer peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 4.0 and 5.5, in the suspension constituted as directed in the labeling.

**Water Determination** (921): not more than 1.5%.

**Assay**—

*Mobile phase, Diluent, and Chromatographic system*—Prepare as directed in the *Assay* under *Cefpodoxime Proxetil*.

*Standard preparation*—Transfer about 30 mg of USP Cefpodoxime Proxetil RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass through a filter having a 0.45- $\mu$ m or finer porosity.

*Assay preparation*—Constitute a container of Cefpodoxime Proxetil for Oral Suspension as directed in the labeling. Shake the resulting suspension thoroughly, and determine its density. Transfer an accurately weighed quantity of the suspension, equivalent to about 50 mg of cefpodoxime, to a 100-mL volumetric flask. Add 10 mL of water, and shake to disperse. Add 20 mL of acetonitrile, and sonicate for 15 minutes. Cool to room temperature, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45- $\mu$ m or finer porosity.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in the portion of Oral Suspension taken by the formula:

$$2CP(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cefpodoxime Proxetil RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g per mg, of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in USP Cefpodoxime Proxetil RS; and  $r_U$  and  $r_S$  are the sums of the peak responses for cefpodoxime proxetil S-epimer and cefpodoxime proxetil R-epimer obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefpodoxime Proxetil Tablets

» Cefpodoxime Proxetil Tablets contain an amount of Cefpodoxime Proxetil equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ).

**Packaging and storage**—Preserve in tight containers, at controlled room temperature.

**USP Reference standards** (11)—  
USP Cefpodoxime Proxetil RS

**Identification**—The retention times of the cefpodoxime proxetil R-epimer peak and the cefpodoxime proxetil S-epimer peak in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium*—Dissolve 54.5 g of glycine and 42.6 g of sodium chloride in about 500 mL of water in a 1000-mL volumetric flask. Cautiously add, with swirling, 14.2 mL of hydrochloric acid, and allow to cool. Dilute with water to volume, and mix. Transfer 50 mL of this stock solution to a flask, and dilute with water to 900 mL to obtain a solution having a pH of  $3.0 \pm 0.1$ . [NOTE—If necessary, adjust the pH of the stock solution with 10 N sodium hydroxide so that when 50 mL is diluted with water to 900 mL the pH of the *Dissolution Medium* is  $3.0 \pm 0.1$ .]

*Apparatus 2*: 75 rpm.

*Time*: 30 minutes.

*Procedure*—Determine the amount of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) dissolved by employing UV absorption at about 259 nm on filtered portions of the solution under test in comparison with a *Standard* solution having a known concentration of USP Cefpodoxime Proxetil RS prepared by dissolving an accurately weighed portion in a small volume of methanol and diluting quantitatively with *Dissolution Medium*.

**Tolerances**—Not less than 70% (Q) of the labeled amount of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water Determination** (921): not more than 5.0%.

**Assay**—

*Mobile phase, Diluent, and Chromatographic system*—Prepare as directed in the *Assay* under *Cefpodoxime Proxetil*.

*Standard preparation*—Transfer about 30 mg of USP Cefpodoxime Proxetil RS, accurately weighed, to a 50-mL volumetric flask, dissolve in 5 mL of methanol, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. Pass through a filter having a 0.45- $\mu$ m or finer porosity.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of cefpodoxime to a 100-mL volumetric flask. Dissolve in 40 mL of *Diluent*, sonicate for 5 minutes. Cool to room temperature, dilute with *Diluent* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, dilute with *Diluent* to volume, mix, and pass through a filter having a 0.45- $\mu$ m or finer porosity.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-

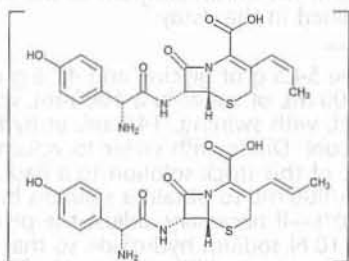


tity, in mg of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in the portion of Tablets taken by the formula:

$$2CP(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cefpodoxime Proxetil RS in the *Standard preparation*; P is the designated potency, in  $\mu\text{g}$  per mg, of cefpodoxime ( $C_{15}H_{17}N_5O_6S_2$ ) in USP Cefpodoxime Proxetil RS; and  $r_U$  and  $r_S$  are the sums of the peak responses for cefpodoxime proxetil S-epimer and cefpodoxime proxetil R-epimer obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefprozil



$C_{18}H_{19}N_3O_5S \cdot H_2O$  407.44  
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[amino(4-hydroxyphenyl)acetyl]amino]-8-oxo-3-(1-propenyl)-, monohydrate, [6R-[6 $\alpha$ ,7 $\beta$ (R\*)]]-; (6R,7R)-7-[(R)-2-Amino-2-(p-hydroxyphenyl)acetamido]-8-oxo-3-propenyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate [121123-17-9].  
Anhydrous 389.43  
[92665-29-7].

### DEFINITION

Cefprozil contains NLT 900  $\mu\text{g}$  and NMT 1050  $\mu\text{g}/\text{mg}$  of cefprozil ( $C_{18}H_{19}N_3O_5S$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**  
Standard: USP Cefprozil RS  
Acceptance criteria: Meets the requirements
- **B.** The retention times of the cefprozil (Z)-isomer and cefprozil (E)-isomer peaks from the *Sample solution* correspond to those of the *Standard solutions*, as obtained in the *Assay*.

### ASSAY

- **PROCEDURE**  
Buffer: 11.5 g/L of monobasic ammonium phosphate in water. Adjust, if necessary, with phosphoric acid to a pH of 4.4.  
Mobile phase: Acetonitrile and Buffer (100:900)  
System suitability solution: 0.125 mg/mL each of USP Cefprozil (Z)-isomer RS and USP Cefprozil (E)-isomer RS in water. Use this solution within 6 h.  
Standard solution 1: 0.25 mg/mL of USP Cefprozil (Z)-isomer RS in water. Use this solution within 6 h.  
Standard solution 2: 0.025 mg/mL of USP Cefprozil (E)-isomer RS in water. Use this solution within 6 h.  
Sample solution: 0.3 mg/mL of Cefprozil in water. Shake to dissolve. Use this solution within 6 h.  
Chromatographic system  
(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm  $\times$  30-cm; 5- $\mu\text{m}$  packing L1

Flow rate: 1 mL/min

Injection volume: 10  $\mu\text{L}$

### System suitability

Samples: System suitability solution and Standard solution 1

[NOTE—The relative retention times for cefprozil (Z)-isomer and cefprozil (E)-isomer are about 0.7 and 1.0, respectively.]

### Suitability requirements

Resolution: NLT 2.5 between cefprozil (Z)-isomer and cefprozil (E)-isomer, System suitability solution

Tailing factor: 0.9–1.1, Standard solution 1

Relative standard deviation: NMT 2.0%, Standard solution 1

### Analysis

Samples: Standard solution 1, Standard solution 2, and Sample solution

Calculate the amount ( $\mu\text{g}$ ) of cefprozil (Z)-isomer ( $C_{18}H_{19}N_3O_5S$ ) in each mg of Cefprozil taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times P$$

$r_U$  = peak response of cefprozil (Z)-isomer from the Sample solution

$r_S$  = peak response of cefprozil (Z)-isomer from Standard solution 1

$C_S$  = concentration of USP Cefprozil (Z)-isomer RS in Standard solution 1 (mg/mL)

$C_U$  = concentration of Cefprozil in the Sample solution (mg/mL)

P = potency of USP Cefprozil (Z)-isomer RS ( $\mu\text{g}/\text{mg}$ )

Calculate the amount ( $\mu\text{g}$ ) of cefprozil (E)-isomer ( $C_{18}H_{19}N_3O_5S$ ) in each mg of Cefprozil taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times P$$

$r_U$  = peak response of cefprozil (E)-isomer from the Sample solution

$r_S$  = peak response of cefprozil (E)-isomer from Standard solution 2

$C_S$  = concentration of USP Cefprozil (E)-isomer RS in Standard solution 2 (mg/mL)

$C_U$  = concentration of Cefprozil in the Sample solution (mg/mL)

P = potency of USP Cefprozil (E)-isomer RS ( $\mu\text{g}/\text{mg}$ )

Calculate the quantity, in  $\mu\text{g}$ , of cefprozil ( $C_{18}H_{19}N_3O_5S$ ) in each mg of Cefprozil taken by adding the values, in  $\mu\text{g}/\text{mg}$ , of the cefprozil (Z)-isomer and the cefprozil (E)-isomer.

Acceptance criteria: 900–1050  $\mu\text{g}/\text{mg}$  on the anhydrous basis

### IMPURITIES

#### • ORGANIC IMPURITIES, PROCEDURE 1

Use *Organic Impurities, Procedure 1* when the impurity profile includes Z-cefprozil open ring, E-cefprozil open ring, and cefprozil related compound K.

Solution A: 11.5 g/L of monobasic ammonium phosphate in water. Adjust, if necessary, with phosphoric acid or ammonium hydroxide to a pH of 4.4.

Solution B: Acetonitrile and Solution A (1:1)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	81	19
8	81	19
20	36	64



Table 1 (Continued)

Time (min)	Solution A (%)	Solution B (%)
25	36	64
27	81	19
30	81	19

[NOTE—These gradient elution times are established on an HPLC system with a dwell volume of approximately 1.3 mL. The gradient elution times in the table can be adjusted as necessary to achieve the separation described.]

**Standard stock solution:** 0.25 mg/mL each of USP Cefprozil (Z)-Isomer RS, USP Amoxicillin Related Compound I RS, and USP Cefprozil Related Compound D RS in a mixture of 1 M hydrochloric acid and *Solution A*. Prepare the solution as follows. Dissolve USP Amoxicillin Related Compound I RS, USP Cefprozil (Z)-Isomer RS, and USP Cefprozil Related Compound D RS in 1 M hydrochloric acid, using 20% of the final volume. Dilute with *Solution A* to volume.

**Sensitivity solution:** 2.5 µg/mL each of cefprozil (Z)-isomer, amoxicillin related compound I, and cefprozil related compound D in *Solution A* from *Standard stock solution*. Store the solution at 4°, and use within 8 h.

**Standard solution:** 50 µg/mL each of cefprozil (Z)-isomer, amoxicillin related compound I, and cefprozil related compound D in *Solution A* from the *Standard stock solution*. Store the solution at 4°, and use within 12 h.

**Sample solution:** 5 mg/mL of Cefprozil in a mixture of 1 M hydrochloric acid and *Solution A*, prepared as follows. Dissolve the Cefprozil first in 1 M hydrochloric acid using 4% of the final volume, and then dilute with *Solution A* to volume. Store the solution at 4°, and use within 3 h.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Temperatures**

**Column:** 40°

**Autosampler:** 4°

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

#### System suitability

**Samples:** *Sensitivity solution* and *Standard solution*

[NOTE—USP Cefprozil Related Compound D RS contains the (Z)- and (E)-isomers of cefprozil related compound D. See *Table 2* for relative retention times.]

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amoxicillin related compound I <sup>a</sup>	0.40	0.3
Cefadroxil	0.54	0.5
Hydroxyphenyldiketopiperazine <sup>b</sup>	0.61	0.3
Cefprozil related compound D (Z)-isomer <sup>c,d</sup>	0.69	0.3
Cefprozil related compound D (E)-isomer <sup>e</sup>	0.91	
O-Acyl cefprozil <sup>f</sup>	0.76	
Cefprozil (Z)-isomer	1.0	
Cefprozil (E)-isomer	1.37	—
Z-Cefprozil open ring <sup>g</sup>	1.74	0.2
Cefprozil related compound H (Z)-isomer <sup>h,i</sup>	1.95	0.2
Cefprozil related compound H (E)-isomer <sup>i</sup>	2.19	
E-Cefprozil open ring <sup>k</sup>	2.08	0.2
Cefprozil related compound K <sup>l,m</sup>	2.76	0.1
	2.86	0.1
	2.91	0.1
	3.01	0.1
Any individual unspecified impurity	—	0.1
Total impurities	—	2.0

<sup>a</sup> (R)-2-Amino-2-(4-hydroxyphenyl)acetic acid.

<sup>b</sup> 3-(Aminomethylene)-6-(4-hydroxyphenyl)piperazine-2,5-dione.

<sup>c</sup> 7-Amino-3-propenylcephalosporanic acid (Z-isomer); (6R,7R)-7-Amino-8-oxo-3-[(Z)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>d</sup> The sum of the two isomers is reported. The limit for the sum is 0.3%.

<sup>e</sup> 7-Amino-3-propenylcephalosporanic acid (E-isomer); (6R,7R)-7-Amino-8-oxo-3-[(E)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> (6R,7R)-7-[(R)-2-Amino-2-[(R)-2-amino-2-(4-hydroxyphenyl)acetoxylphenyl]acetamido]-8-oxo-3-[(Z)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>g</sup> (R)-2-[(R)-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxyl)methyl]-5-[(Z)-prop-1-enyl]-3,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.

<sup>h</sup> N-Acyl cefprozil (Z-isomer); (6R,7R)-7-[(R)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-8-oxo-3-[(Z)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>i</sup> The sum of the two isomers is reported. The limit for the sum is 0.2%.

<sup>j</sup> N-Acyl cefprozil (E-isomer); (6R,7R)-7-[(R)-2-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-8-oxo-3-[(E)-prop-1-enyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>k</sup> (R)-2-[(R)-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxyl)methyl]-5-[(E)-prop-1-enyl]-3,6-dihydro-2H-1,3-thiazine-4-carboxylic acid.

<sup>l</sup> Hydroxyphenyldiketopiperazine lactone; 3-(5-Ethyl-7-oxo-2,4,5,7-tetrahydro-1H-furo[3,4-d][1,3]thiazin-2-yl)-6-(4-hydroxyphenyl)piperazine-2,5-dione.

<sup>m</sup> The system resolves four isomers of cefprozil related compound K.

#### Suitability requirements

**Resolution:** NLT 1.4 between the (E)-isomer of cefprozil related compound D and cefprozil (Z)-isomer, *Standard solution*

**Relative standard deviation:** NMT 10.0% for cefprozil, amoxicillin related compound I, and each isomer of cefprozil related compound D, *Standard solution*

**Signal-to-noise ratio:** NLT 10 for cefprozil, amoxicillin related compound I, and each isomer of cefprozil related compound D, *Sensitivity solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of amoxicillin related compound I in the portion of Cefprozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$



- $r_U$  = peak response of amoxicillin related compound I from the *Sample solution*  
 $r_S$  = peak response of amoxicillin related compound I from the *Standard solution*  
 $C_S$  = concentration of USP Amoxicillin Related Compound I RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Cefprozil in the *Sample solution* (mg/mL)  
 $P$  = potency of amoxicillin related compound I in USP Amoxicillin Related Compound I RS (mg/mg)  
 Calculate the percentage of cefprozil related compound D in the portion of Cefprozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

- $r_U$  = sum of the responses for cefprozil related compound D (Z)-isomer and cefprozil related compound D (E)-isomer from the *Sample solution*  
 $r_S$  = peak response of cefprozil related compound D (Z)-isomer from the *Standard solution*  
 $C_S$  = concentration of USP Cefprozil Related Compound D RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Cefprozil in the *Sample solution* (mg/mL)  
 $P$  = potency of cefprozil related compound D (Z)-isomer in USP Cefprozil Related Compound D RS (mg/mg)  
 Calculate the percentage of each of the other impurities in the portion of Cefprozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

- $r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = peak response of cefprozil from the *Standard solution*  
 $C_S$  = concentration of USP Cefprozil (Z)-isomer RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Cefprozil in the *Sample solution* (mg/mL)  
 $P$  = potency of USP Cefprozil (Z)-isomer RS (mg/mg)  
**Acceptance criteria:** See Table 2. The reporting threshold is 0.05%.

#### • ORGANIC IMPURITIES, PROCEDURE 2

Use *Organic Impurities, Procedure 2* when the impurity profile includes ethoxycarbonyl cefprozil, methoxycefadroxil, cefprozil delta-3 isomer, cefprozil amide, and cefprozil dimer.

**Solution A:** 4 g/L of monobasic sodium phosphate adjusted with dilute phosphoric acid (1 in 10) to a pH of  $4.2 \pm 0.05$

**Solution B:** Acetonitrile and Solution A (1:1)

**Mobile phase:** See Table 3.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	95	5
20	70	30
40	40	60
50	0	100

Table 3 (Continued)

Time (min)	Solution A (%)	Solution B (%)
60	0	100
62	95	5
70	95	5

**Diluent:** 0.85 g/L of monobasic potassium phosphate and 1.16 g/L of anhydrous dibasic sodium phosphate in water

**System suitability stock solution:** 0.15 mg/mL of USP Cefadroxil RS and 0.75 mg/mL of USP Cefprozil Related Compound D RS, prepared as follows. Dissolve USP Cefadroxil RS in *Solution A*, using 20% of the final volume. Add USP Cefprozil Related Compound D RS, mix, and dilute with *Diluent* to volume.

**System suitability solution:** 15 µg/mL of USP Cefadroxil RS and 75 µg/mL of USP Cefprozil Related Compound D RS from the *System suitability stock solution* and 1.5 mg/mL of USP Cefprozil RS in *Solution A*  
**Standard solution:** 15 µg/mL of USP Cefprozil RS in *Solution A*

**Sample solution:** 1.5 mg/mL of Cefprozil in *Solution A*. Refrigerate the solution, and use within 1 h.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

#### Temperatures

**Column:** NMT 30°

**Autosampler:** 4°

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between the (Z)-isomer of cefprozil related compound D and cefadroxil; NLT 1.5 between cefadroxil and the (E)-isomer of cefprozil related compound D, *System suitability solution*

**Relative standard deviation:** NMT 5.0% for the sum of the cefprozil (Z)-isomer and cefprozil (E)-isomer, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cefprozil taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (1/F) \times 100$$

- $r_U$  = peak response of each impurity from the *Sample solution*  
 $r_S$  = sum of the responses for cefprozil (Z)-isomer and cefprozil (E)-isomer from the *Standard solution*  
 $C_S$  = concentration of USP Cefprozil RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Cefprozil in the *Sample solution* (mg/mL)  
 $P$  = potency of USP Cefprozil RS (mg/mg)  
 $F$  = relative response factor (see Table 4)

**Acceptance criteria:** See Table 4. The reporting threshold is 0.05%.



Table 4

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound I <sup>a</sup>	0.17	1.5	0.15
Cefprozil related compound D (Z)-isomer <sup>b</sup>	0.57	0.56	0.30
Cefadroxil	0.62	1.1	1.0
Methoxycefadroxil <sup>c</sup>	0.65	0.44	0.15
Cefprozil related compound D (E)-isomer <sup>d</sup>	0.73	0.56	0.30
Cefprozil delta-3 isomer <sup>e</sup>	0.92	0.95	0.2
Cefprozil (Z)-isomer	1.0	—	—
Cefprozil (E)-isomer	1.17	—	—
Cefprozil related compound H <sup>f</sup>	1.33	0.93	0.15
Cefprozil amide <sup>g</sup>	1.46	0.90	0.15
Ethoxycarbonylcefprozil <sup>h</sup>	2.08	0.70	0.15
Cefprozil dimer <sup>i</sup>	2.21	0.90	0.2
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	2.00

<sup>a</sup> (R)-2-Amino-2-(4-hydroxyphenyl)acetic acid.

<sup>b</sup> 7-Amino-3-propenylcephalosporanic acid (Z-isomer); (6R,7R)-7-Amino-8-oxo-3-[(Z)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>c</sup> (6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-3-(methoxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>d</sup> 7-Amino-3-propenylcephalosporanic acid (E-isomer); (6R,7R)-7-Amino-8-oxo-3-[(E)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>e</sup> (6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-8-oxo-3-[(Z)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>f</sup> N-Acyl cefprozil (Z-isomer); (6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-2-(4-hydroxyphenyl)acetamido]-8-oxo-3-[(Z)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>g</sup> (R)-2-[(6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-8-oxo-3-[(Z)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamido]-2-(4-hydroxyphenyl)acetic acid.

<sup>h</sup> (6R,7R)-7-[(R)-2-Amino-2-(4-ethoxycarbonyloxy)phenyl]acetamido]-8-oxo-3-[(Z)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>i</sup> (6R,7R)-7-[(R)-2-[(6R,7R)-7-[(R)-2-Amino-2-(4-hydroxyphenyl)acetamido]-8-oxo-3-[(Z)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxamido]-2-(4-hydroxyphenyl)acetamido]-8-oxo-3-[(Z)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

## SPECIFIC TESTS

- **CRYSTALLINITY** (695): Meets the requirements

- **PH** (791)

Sample solution: 5 mg/mL in water

Acceptance criteria: 3.5–6.5

- **WATER DETERMINATION, Method I** (921): 3.5%–6.5%

- **CEFPROZIL (E)-ISOMER RATIO**

Buffer, Mobile phase, System suitability solution, Standard solution 1, Standard solution 2, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

### Analysis

Samples: Standard solution 1, Standard solution 2, and Sample solution

Calculate the ratio of the cefprozil (E)-isomer to total cefprozil in the portion of Cefprozil taken:

$$\text{Result} = E/(E + Z)$$

E = amount of cefprozil (E)-isomer as determined in the Assay (μg/mg)

Z = amount of cefprozil (Z)-isomer as determined in the Assay (μg/mg)

Acceptance criteria: The ratio is 0.06–0.11.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS** (11)
  - USP Amoxicillin Related Compound I RS
  - (R)-2-Amino-2-(4-hydroxyphenyl)acetic acid.  
C<sub>8</sub>H<sub>9</sub>NO<sub>3</sub> · 167.16
  - USP Cefadroxil RS
  - USP Cefprozil RS
  - USP Cefprozil (E)-Isomer RS
  - USP Cefprozil (Z)-Isomer RS
  - USP Cefprozil Related Compound D RS
  - 7-Amino-3-propenylcephalosporanic acid; (6R,7R)-7-Amino-8-oxo-3-[(Z)-prop-1-en-1-yl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.  
C<sub>10</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>S · 240.28

## Cefprozil for Oral Suspension

### DEFINITION

Cefprozil for Oral Suspension is a dry mixture of Cefprozil and one or more suitable buffers, flavors, preservatives, suspending agents, and sweeteners. It contains NLT 90.0% and NMT 120.0% of the labeled amount of cefprozil (C<sub>18</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>S).

### IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHY**

Diluent: Acetone and 0.1 N hydrochloric acid (4:1)

Standard solution: 5 mg/mL of USP Cefprozil (Z)-Isomer RS in Diluent

Sample solution: Nominally 5 mg/mL of cefprozil in Diluent from Cefprozil for Oral Suspension. Shake for 5 min, and allow to settle. Use the supernatant.

### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 μL

Developing solvent system: Butyl alcohol, glacial acetic acid, and water (60:20:20)

### Analysis

Samples: Standard solution and Sample solution

Allow the spots to dry, and develop the chromatogram in an equilibrated chamber with the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate, and allow it to air-dry in a hood. Place the dry plate in a chamber containing iodine vapors. Examine the plate, and locate the spots.

Acceptance criteria: The *R<sub>f</sub>* value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

- **B.** The retention times of the cefprozil (Z)-isomer and cefprozil (E)-isomer peaks of the *Sample solution* correspond to those of *Standard solution 1* and *Standard solution 2*, respectively, as obtained in the Assay.

### ASSAY

- **PROCEDURE**

Buffer: 11.5 g/L of monobasic ammonium phosphate in water. Adjust, if necessary, with phosphoric acid to a pH of 4.4.

Mobile phase: Acetonitrile and Buffer (100:900).

[NOTE—Decreasing the proportion of acetonitrile increases retention times and improves the resolution between the cefprozil isomer peaks.]



**Standard solution 1:** 0.25 mg/mL of USP Cefprozil (Z)-Isomer RS. Use this solution within 6 h.

**Standard stock solution:** 0.25 mg/mL of USP Cefprozil (E)-Isomer RS

**Standard solution 2:** 0.025 mg/mL of USP Cefprozil (E)-Isomer RS in water from the *Standard stock solution*. Use this solution within 6 h.

**System suitability solution:** A mixture of equal volumes of *Standard solution 1* and the *Standard stock solution*. Use this solution within 6 h.

**Sample stock solution:** Nominally 1 mg/mL of cefprozil in water from Cefprozil for Oral Suspension. Prepare as follows. Constitute one container of Cefprozil for Oral Suspension as directed in the labeling. Transfer a suitable aliquot, freshly mixed and free from air bubbles, to a volumetric flask, dilute with water to volume, and mix, sonicating briefly.

**Sample solution:** Nominally 0.3 mg/mL of cefprozil in water from the *Sample stock solution*. Pass a portion of this solution through a filter of 0.5- $\mu$ m or finer pore size. Use this solution within 6 h.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Samples:** *Standard solution 1* and *System suitability solution*

[NOTE—The relative retention times for the cefprozil (Z)-isomer and the cefprozil (E)-isomer are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between cefprozil (Z)-isomer and cefprozil (E)-isomer, *System suitability solution*

**Column efficiency:** NLT 2500 theoretical plates, cefprozil (Z)-isomer, *Standard solution 1*

Calculate as follows:

$$\text{Result} = (t_R/W_{h/2})^2 \times 5.545$$

$t_R$  = retention time of cefprozil (Z)-isomer

$W_{h/2}$  = peak width at half-height

**Tailing factor:** 0.9–1.1, cefprozil (Z)-isomer, *Standard solution 1*

Calculate as follows:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$  = width of the peak at 10% height

$f$  = distance from the peak maximum to the leading edge of the peak measured at 10% of the peak height

**Relative standard deviation:** NMT 2.0%, *Standard solution 1*

#### Analysis

**Samples:** *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the concentration, in mg/mL, of the cefprozil (Z)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

$r_U$  = peak response of the cefprozil (Z)-isomer from the *Sample solution*

$r_S$  = peak response of the cefprozil (Z)-isomer from *Standard solution 1*

$C_S$  = concentration of USP Cefprozil (Z)-Isomer RS in *Standard solution 1* (mg/mL)

$P$  = potency of the cefprozil (Z)-isomer in USP Cefprozil (Z)-Isomer RS ( $\mu$ g/mg)

$F$  = correction factor, 0.001 mg/ $\mu$ g

Calculate the concentration, in mg/mL, of the cefprozil (E)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

$r_U$  = peak response of the cefprozil (E)-isomer from the *Sample solution*

$r_S$  = peak response of the cefprozil (E)-isomer from *Standard solution 2*

$C_S$  = concentration of USP Cefprozil (E)-Isomer RS in *Standard solution 2* (mg/mL)

$P$  = potency of the cefprozil (E)-isomer in USP Cefprozil (E)-Isomer RS ( $\mu$ g/mg)

$F$  = correction factor, 0.001 mg/ $\mu$ g

Calculate the percentage of the labeled amount of cefprozil ( $C_{18}H_{19}N_3O_5S$ ) in the portion of Cefprozil for Oral Suspension taken:

$$\text{Result} = [(C_Z + C_E)/C_U] \times 100$$

$C_Z$  = concentration of the cefprozil (Z)-isomer in the *Sample solution* (mg/mL)

$C_E$  = concentration of the cefprozil (E)-isomer in the *Sample solution* (mg/mL)

$C_U$  = nominal concentration of cefprozil in the *Sample solution* (mg/mL)

Acceptance criteria: 90%–120.0%

#### PERFORMANCE TESTS

##### • UNIFORMITY OF DOSAGE UNITS (905)

For solids packaged in single-unit containers: Meets the requirements

##### • DELIVERABLE VOLUME (698)

For solids packaged in multiple-unit containers: Meets the requirements

#### SPECIFIC TESTS

##### • PH (791)

**Sample solution:** Constitute Cefprozil for Oral Suspension as directed in the labeling.

Acceptance criteria: 4.0–6.0

##### • WATER DETERMINATION, Method I (921): NMT 3.0%

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE: Preserve in tight containers.

##### • USP REFERENCE STANDARDS (11)

USP Cefprozil (E)-Isomer RS

USP Cefprozil (Z)-Isomer RS

## Cefprozil Tablets

#### DEFINITION

Cefprozil Tablets contain NLT 90.0% and NMT 120.0% of the labeled amount of cefprozil ( $C_{18}H_{19}N_3O_5S$ ).

#### IDENTIFICATION

##### • A. THIN-LAYER CHROMATOGRAPHY

**Diluent:** Acetone and 0.1 N hydrochloric acid (4:1)

**Standard solution:** 5 mg/mL of USP Cefprozil (Z)-Isomer RS in *Diluent*

**Sample solution:** Nominally 2.5 mg/mL of cefprozil from 1 Tablet in *Diluent*. Shake for 5 min, and allow the mixture to settle. Use the supernatant.

##### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (60:20:20)



**Analysis**

**Samples:** *Standard solution* and *Sample solution*.

Allow the spots to dry, and develop the chromatogram in an equilibrated chamber with the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate, and allow it to air-dry in a hood. Place the dry plate in a chamber containing iodine vapors. Examine the plate, and locate the spots.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

- B.** The retention times of the cefprozil (Z)-isomer and cefprozil (E)-isomer peaks of the *Sample solution* correspond to those of *Standard solution 1* and *Standard solution 2*, respectively, as obtained in the Assay.

**ASSAY****PROCEDURE**

**Buffer:** 11.5 g/L of monobasic ammonium phosphate in water. Adjust, if necessary, with phosphoric acid to a pH of 4.4.

**Mobile phase:** Acetonitrile and *Buffer* (100:900).

[NOTE—Decreasing the proportion of acetonitrile increases retention times and improves the resolution between the cefprozil isomer peaks.]

**Standard solution 1:** 0.25 mg/mL of USP Cefprozil (Z)-Isomer RS. Use this solution within 6 h.

**Standard stock solution:** 0.25 mg/mL of USP Cefprozil (E)-Isomer RS

**Standard solution 2:** 0.025 mg/mL of USP Cefprozil (E)-Isomer RS in water from the *Standard stock solution*. Use this solution within 6 h.

**System suitability solution:** A mixture of equal volumes of *Standard solution 1* and the *Standard stock solution*. Use this solution within 6 h.

**Sample stock solution:** Nominally 6 mg/mL of cefprozil in water from Tablets, prepared as follows. Transfer a suitable number of Tablets to a volumetric flask containing water. Allow the Tablets to disintegrate with the aid of swirling and sonication. Dilute with water to volume.

**Sample solution:** Nominally 0.3 mg/mL of cefprozil in water from the *Sample stock solution*. Pass a portion of this solution through a filter of 0.5- $\mu$ m or finer pore size. Use this solution within 6 h.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

**System suitability**

**Samples:** *Standard solution 1* and *System suitability solution*

[NOTE—The relative retention times for cefprozil (Z)-isomer and cefprozil (E)-isomer are about 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.5 between cefprozil (Z)-isomer and cefprozil (E)-isomer, *System suitability solution*

**Column efficiency:** NLT 2500 theoretical plates, cefprozil (Z)-isomer, *Standard solution 1*  
Calculate as follows:

$$\text{Result} = (t_R/W_{h/2})^2 \times 5.545$$

$t_R$  = retention time of cefprozil (Z)-isomer

$W_{h/2}$  = peak width at half-height

**Tailing factor:** 0.9–1.1, cefprozil (Z)-isomer, *Standard solution 1*

Calculate as follows:

$$\text{Result} = W_{0.1}/2f$$

$W_{0.1}$  = width of the peak at 10% height

$f$  = distance from the peak maximum to the leading edge of the peak measured at 10% of the peak height

**Relative standard deviation:** NMT 2.0%, *Standard solution 1*

**Analysis**

**Samples:** *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the concentration, in mg/mL, of the cefprozil (Z)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

$r_U$  = peak response of the cefprozil (Z)-isomer from the *Sample solution*

$r_S$  = peak response of the cefprozil (Z)-isomer from *Standard solution 1*

$C_S$  = concentration of USP Cefprozil (Z)-Isomer RS in *Standard solution 1* (mg/mL)

$P$  = potency of the cefprozil (Z)-isomer in USP Cefprozil (Z)-Isomer RS ( $\mu$ g/mg)

$F$  = correction factor, 0.001 mg/ $\mu$ g

Calculate the concentration, in mg/mL, of the cefprozil (E)-isomer in the *Sample solution*:

$$\text{Result} = (r_U/r_S) \times C_S \times P \times F$$

$r_U$  = peak response of the cefprozil (E)-isomer from the *Sample solution*

$r_S$  = peak response of the cefprozil (E)-isomer from *Standard solution 2*

$C_S$  = concentration of USP Cefprozil (E)-Isomer RS in *Standard solution 2* (mg/mL)

$P$  = potency of the cefprozil (E)-isomer in USP Cefprozil (E)-Isomer RS ( $\mu$ g/mg)

$F$  = correction factor, 0.001 mg/ $\mu$ g

Calculate the percentage of the labeled amount of cefprozil ( $C_{18}H_{19}N_3O_5S$ ) in the portion of Tablets taken:

$$\text{Result} = [(C_Z + C_E)/C_U] \times 100$$

$C_Z$  = concentration of the cefprozil (Z)-isomer in the *Sample solution* (mg/mL)

$C_E$  = concentration of the cefprozil (E)-isomer in the *Sample solution* (mg/mL)

$C_U$  = nominal concentration of cefprozil in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–120.0%

**PERFORMANCE TESTS****DISSOLUTION (711)**

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Mobile phase, Standard solution 1, Standard solution 2, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Sample solution:** Pass the solution under test through a suitable filter. Dilute, if necessary, to 0.3 mg/mL of cefprozil.

**Analysis**

**Samples:** *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Calculate the quantity, in mg, of cefprozil (Z)-isomer dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times P \times F$$

$r_U$  = peak response of cefprozil (Z)-isomer from the *Sample solution*

$r_S$  = peak response of cefprozil (Z)-isomer from *Standard solution 1*

$C_S$  = concentration of USP Cefprozil (Z)-Isomer RS in *Standard solution 1* (mg/mL)



- $D$  = dilution factor of the *Sample solution*  
 $V$  = volume of *Medium*, 900 mL  
 $P$  = potency of the cefprozil (Z)-isomer in USP Cefprozil (Z)-isomer RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = correction factor, 0.001  $\text{mg}/\mu\text{g}$   
 Calculate the quantity, in mg, of cefprozil (E)-isomer dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times D \times V \times P \times F$$

- $r_u$  = peak response of cefprozil (E)-isomer from the *Sample solution*  
 $r_s$  = peak response of cefprozil (E)-isomer from *Standard solution 2*  
 $C_s$  = concentration of USP Cefprozil (E)-isomer RS in *Standard solution 2* ( $\text{mg}/\text{mL}$ )  
 $D$  = dilution factor of the *Sample solution*  
 $V$  = volume of *Medium*, 900 mL  
 $P$  = potency of the cefprozil (E)-isomer in USP Cefprozil (E)-isomer RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = correction factor, 0.001  $\text{mg}/\mu\text{g}$   
 Calculate the percentage of cefprozil ( $\text{C}_{18}\text{H}_{19}\text{N}_3\text{O}_5\text{S}$ ) dissolved:

$$\text{Result} = (M_z + M_e) \times 100/L$$

- $M_z$  = quantity of cefprozil (Z)-isomer dissolved (mg)  
 $M_e$  = quantity of cefprozil (E)-isomer dissolved (mg)  
 $L$  = label claim of cefprozil (mg/Tablet)  
 Tolerances: NLT 75% (Q) of the labeled amount of cefprozil ( $\text{C}_{18}\text{H}_{19}\text{N}_3\text{O}_5\text{S}$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

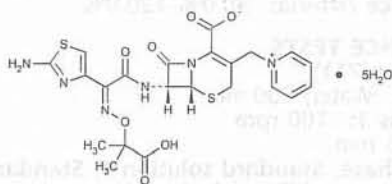
#### SPECIFIC TESTS

- **WATER DETERMINATION**, Method I (921): NMT 7.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Cefprozil (E)-isomer RS  
 USP Cefprozil (Z)-isomer RS

### Ceftazidime



$\text{C}_{22}\text{H}_{22}\text{N}_6\text{O}_7\text{S}_2 \cdot 5\text{H}_2\text{O}$  636.65

Pyridinium, 1-[[[7-[[[(2-amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-hydroxide, inner salt, pentahydrate, [6R[6 $\alpha$ ,7 $\beta$ (Z)]]-1-[[[(6R,7R)-7-[2-(2-amino-4-thiazolyl)glyoxylamido]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]pyridinium hydroxide, inner salt, 7 $^2$ -(Z)-[O(1-carboxy-1-methylethyl)oxime], pentahydrate [78439-06-2].

Anhydrous 546.59

» Ceftazidime contains not less than 95.0 percent and not more than 102.0 percent of  $\text{C}_{22}\text{H}_{22}\text{N}_6\text{O}_7\text{S}_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.

#### USP Reference standards (11)—

USP Ceftazidime Delta-3-Isomer RS  
 USP Ceftazidime Pentahydrate RS  
 USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftazidime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Crystallinity** (695): meets the requirements.

**Sterility Tests** (71)—Where the label states that it is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use *Fluid A* to each 1000 mL of which has been added 10 g of sodium bicarbonate before sterilization.

**pH** (791): between 3.0 and 4.0, in a solution containing 5 mg per mL.

**Loss on drying** (731)—Dry about 300 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses between 13.0% and 15.0% of its weight.

**Other requirements**—Where the label states that Ceftazidime is sterile or that it must be subjected to further processing during the preparation of injectable or other sterile dosage forms, it meets the requirements for *Bacterial endotoxins* under *Ceftazidime for Injection*.

#### Assay—

**pH 7 Buffer**—Dissolve 42.59 g of anhydrous dibasic sodium phosphate and 27.22 g of monobasic potassium phosphate in water to make 1000 mL of solution.

**Mobile phase**—Mix 40 mL of acetonitrile and 200 mL of pH 7 Buffer, and dilute with water to obtain 2000 mL of solution. Filter, using a filter having a porosity of 1  $\mu\text{m}$  or finer, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Transfer about 29 mg of USP Ceftazidime Pentahydrate RS, accurately weighed, to a 25-mL volumetric flask containing 2.5 mL of pH 7 Buffer, and shake until dissolved. Dilute with water to volume, and mix. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this stock solution to a 50-mL volumetric flask, dilute with water to volume, and mix. This solution contains about 100  $\mu\text{g}$  of ceftazidime ( $\text{C}_{22}\text{H}_{22}\text{N}_6\text{O}_7\text{S}_2$ ) per mL.

**Assay preparation**—Transfer about 115 mg of Ceftazidime, accurately weighed, to a 100-mL volumetric flask containing 10.0 mL of pH 7 Buffer, and shake until dissolved. Dilute with water to volume, and mix. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Resolution solution**—Prepare a solution of USP Ceftazidime, Delta-3-Isomer RS in pH 7 Buffer containing about 0.1 mg per mL. Immediately prior to chromatography, mix 1 mL of this solution with 8 mL of water and 1 mL of the stock solution used to prepare the *Standard preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu\text{m}$  packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between ceftazidime and ceftazidime, delta-3-isomer is not less than 2.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the tailing factor for the



analyte peak is not less than 0.75 and not more than 1.5, and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{22}N_6O_7S_2$  in the portion of Ceftazidime taken by the formula:

$$C(r_U / r_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ceftazidime Injection

» Ceftazidime Injection is a sterile isoosmotic solution of Ceftazidime in Water for Injection. It contains one or more suitable buffers and a tonicity-adjusting agent. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{22}H_{22}N_6O_7S_2$ .

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—  
USP Ceftazidime Delta-3-Isomer RS  
USP Ceftazidime Pentahydrate RS  
USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftazidime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.1 USP Endotoxin Unit per mg of ceftazidime.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.0 and 7.5.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

### Assay—

*pH 7 Buffer*, *Mobile phase*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the *Assay under Ceftazidime*.

**Assay preparation**—Allow a container of the Injection to thaw, and mix the solution. Transfer an accurately measured volume of the Injection, equivalent to about 50 mg of ceftazidime, to a 50-mL volumetric flask, dilute with *pH 7 buffer* to volume, and mix. Transfer 5.0 mL of this solution to a second 50-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay under Ceftazidime*. Calculate the quantity, in mg, of

$C_{22}H_{22}N_6O_7S_2$  in each mL of the Injection taken by the formula:

$$0.5(C / V)(r_U / r_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and  $r_U$  and  $r_S$  are the ceftazidime peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ceftazidime for Injection

» Ceftazidime for Injection is a sterile mixture of Sterile Ceftazidime and Sodium Carbonate or Arginine. It contains not less than 90.0 percent and not more than 105.0 percent of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) on the dried and sodium carbonate- or arginine-free basis, and not less than 90.0 percent and not more than 120.0 percent of the labeled amount of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ).

### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for constitution* (CN 1-May-2017), protected from light.

**USP Reference standards** (11)—  
USP L-Arginine RS  
USP Ceftazidime Delta-3-Isomer RS  
USP Ceftazidime Pentahydrate RS  
USP Endotoxin RS

### Identification—

**A:** The chromatograms of the *Assay preparations* exhibit a major peak for ceftazidime, the retention time of which corresponds to that in the chromatogram of the *Standard preparation*.

**B:** It dissolves in 1 N hydrochloric acid with effervescence, evolving a colorless gas, which when passed into *calcium hydroxide TS* produces a white precipitate immediately.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.1 USP Endotoxin Unit per mg of ceftazidime.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.0 and 7.5, in a solution constituted in the sealed container, taking care to relieve the pressure inside the container during constitution, containing 100 mg of ceftazidime per mL.

**Loss on drying** (731)—Dry about 300 mg, accurately weighed, in vacuum at a pressure not exceeding 5 mm of mercury at 25° for 4 hours: where it contains arginine, it loses not more than 12.5% of its weight. Where it contains sodium carbonate, it loses not more than 13.5% of its weight. Where it contains arginine, use the percentage loss obtained, *m*, to calculate, on the dried and arginine-free basis, the result from *Assay preparation 1* obtained as directed in the *Assay*. Where it contains sodium carbonate, heat the residue in vacuum at a pressure not exceeding 5 mm of mercury at 100° an additional 3 hours, and calculate the total percentage of weight loss. Use this percentage, *m*, to calculate, on the dried and sodium carbonate-free basis, the result from *Assay preparation 1* obtained as directed in the *Assay*.



**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Sodium carbonate** (where present)—

**Potassium chloride solution**—Dissolve 19.07 g of potassium chloride in water to make 1000 mL of solution.

**Standard preparation**—Dissolve a suitable quantity of sodium chloride, previously dried at 105° for 2 hours and accurately weighed, in water to obtain a solution having a known concentration of about 14 µg per mL. Transfer 10 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

**Test preparation**—Use the stock solution used to prepare *Assay preparation 1* in the Assay, diluting it quantitatively, and stepwise if necessary, with water to obtain a solution containing about 12.5 µg of sodium carbonate per mL. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add 10.0 mL of *Potassium chloride solution*, dilute with water to volume, and mix.

**Blank solution**—Transfer 10.0 mL of *Potassium chloride solution* to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparation* and the *Test preparation* at the sodium emission line of 589.0 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a sodium hollow-cathode lamp and an air-acetylene flame, using the *Blank solution* as the blank. Calculate the percentage of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) in the portion of Cefazidime for Injection taken by the formula:

$$(105.99/116.88)(0.1C/M)(A_U / A_S)$$

in which 105.99 is the molecular weight of sodium carbonate; 116.88 is twice the molecular weight of sodium chloride; C is the concentration, in µg per mL, of sodium chloride in the *Standard preparation*; M is the quantity, in mg, of Cefazidime for Injection in each mL of the *Test preparation*, based on the quantity taken to prepare the stock solution and the extent of dilution; and  $A_U$  and  $A_S$  are the absorbances of the *Test preparation* and the *Standard preparation*, respectively. Use this percentage to calculate, on the dried and sodium carbonate-free basis, the result from *Assay preparation 1* obtained as directed in the Assay.

**Limit of pyridine**—

**Mobile phase**—Mix 300 mL of acetonitrile and 100 mL of 0.25 M monobasic ammonium phosphate, dilute with water to obtain 1000 mL of solution, and adjust with ammonium hydroxide to a pH of  $7.0 \pm 0.1$ . Pass this solution through a filter having a 1-µm or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**pH 7 Buffer**—Dissolve 5.68 g of anhydrous dibasic sodium phosphate and 3.63 g of monobasic potassium phosphate in water to make 1000 mL of solution.

**Standard solution**—Transfer about 250 mg of pyridine, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix. Immediately prior to chromatography, transfer 2.0 mL of this solution to a 200-mL volumetric flask, dilute with pH 7 Buffer to volume, and mix. This solution contains about 25 µg of pyridine per mL.

**Test solution**—Transfer about 660 mg of Cefazidime for Injection, just removed from its container and accurately weighed, to a 100-mL volumetric flask, promptly add pH 7 buffer to volume, and mix. Store this solution in a cool place, and use it within 1 hour.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate is about 1.6 mL per minute. Chromatograph the *Standard solution*, and record the peak responses

as directed for *Procedure*: the tailing factor for the analyte peak is not more than 2.5; and the relative standard deviation for replicate injections is not more than 3%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas of the responses for the pyridine peaks. Calculate the percentage of pyridine in the portion of Cefazidime for Injection taken by the formula:

$$10(C/W)(r_U / r_S)$$

in which C is the concentration, in µg per mL, of pyridine in the *Standard solution*; W is the weight, in mg, of Cefazidime for Injection taken; and  $r_U$  and  $r_S$  are the pyridine peak responses obtained from the *Test solution* and the *Standard solution*, respectively: not more than 0.4% of pyridine is found where it contains sodium carbonate; and not more than 0.3% where it contains arginine.

**Content of arginine** (where present)—

**Mobile phase**—Dissolve 1.15 g of monobasic ammonium phosphate in about 800 mL of water. Adjust with phosphoric acid to a pH of  $2.0 \pm 0.1$ , dilute with water to 1000 mL, and mix. Prepare a filtered and degassed mixture of acetonitrile and this solution (750:250). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve accurately weighed quantities of USP Cefazidime Pentahydrate RS and USP L-Arginine RS in water to obtain a solution containing known concentrations of about 0.2 mg of each per mL.

**Test preparation**—Quantitatively dissolve an accurately weighed portion of Cefazidime for Injection in water to obtain a solution having a concentration of about 0.2 mg of cefazidime per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 206-nm detector, a 4.6-mm × 50-cm saturator pre-column containing packing L27, and a 4-mm × 25-cm analytical column containing packing L20. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the resolution,  $R$ , between the cefazidime and the arginine peaks is not less than 6.0; and the tailing factor for the arginine peak is not more than 4.0.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of arginine ( $\text{C}_6\text{H}_{14}\text{N}_4\text{O}_2$ ) in the Cefazidime for Injection taken by the formula:

$$100(C_S / C_U)(r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of USP L-Arginine RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of Cefazidime for Injection in the *Test preparation*, based on the weight, in mg, of Cefazidime for Injection taken and the extent of dilution; and  $r_U$  and  $r_S$  are the arginine peak responses obtained from the *Test preparation* and the *Standard preparation*, respectively. Use this percentage to calculate, on the anhydrous and arginine-free basis, the assay result from *Assay preparation 1* obtained as directed in the Assay.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

**Assay**—

pH 7 buffer, *Mobile phase*, *Standard preparation*, *Resolution solution*, and *Chromatographic system*—Proceed as directed in the Assay under Cefazidime.



**Assay preparation 1**—Transfer an accurately weighed quantity of Ceftazidime for Injection, equivalent to about 250 mg of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ), to a 250-mL volumetric flask, dilute with water to volume, and mix to obtain a stock solution. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Assay preparation 2** (where it is represented as being in a single-dose container)—Constitute a container of Ceftazidime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing about 1 mg of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) per mL. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Assay preparation 3** (where the label states the quantity of ceftazidime in a given volume of constituted solution)—Constitute a container of Ceftazidime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with water to obtain a solution containing about 1 mg of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) per mL. [NOTE—Protect this solution from light.] Immediately prior to chromatography, transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed as directed for Procedure in the Assay under Ceftazidime. Calculate the percentage of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) on the dried and sodium carbonate-free or arginine-free basis in the portion of Ceftazidime for Injection taken by the formula:

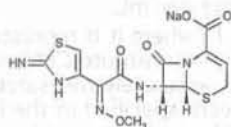
$$25,000[C/[W(100 - m - s)]](r_u / r_s)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) in the Standard preparation;  $W$  is the quantity, in mg, of Ceftazidime for Injection taken to prepare Assay preparation 1;  $m$  is the total percentage of loss on drying;  $s$  is the percentage of sodium carbonate or arginine in the Ceftazidime for Injection taken; and  $r_u$  and  $r_s$  are the peak responses obtained from the Assay preparation and the Standard preparation, respectively. Calculate the quantity, in mg, of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C)(r_u / r_s)$$

in which  $L$  is the labeled quantity, in mg, of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) in the container, or in the volume of constituted solution taken; and  $D$  is the concentration, in  $\mu\text{g}$ , of ceftazidime ( $C_{22}H_{22}N_6O_7S_2$ ) per mL, of Assay preparation 2 or Assay preparation 3, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution.

## Ceftizoxime Sodium



$C_{13}H_{12}N_5NaO_5S_2$  405.38

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(2,3-dihydro-2-imino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxomonosodium salt, [6R-[6 $\alpha$ ,7 $\beta$ (Z)]]-. Sodium (6R,7R)-7-[2-(2-imino-4-thiazolin-4-yl)glyoxylamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 7 $^2$ -(Z)-(O-methyloxime) [68401-82-1].

» Ceftizoxime Sodium contains the equivalent of not less than 850  $\mu\text{g}$  and not more than 995  $\mu\text{g}$  of ceftizoxime ( $C_{13}H_{13}N_5O_5S_2$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Ceftizoxime RS

USP Endotoxin RS

**Identification**—

A: The chromatogram of the Assay preparation obtained as directed in the Assay exhibits a major peak for ceftizoxime, the retention time of which corresponds to that exhibited in the chromatogram of the Standard preparation obtained as directed in the Assay.

B: It responds to the tests for Sodium (191).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 6.0 and 8.0, in a solution (1 in 10).

**Water Determination, Method I** (921): not more than 8.5%.

**Other requirements**—Where the label states that Ceftizoxime Sodium is sterile, it meets the requirements for Sterility and Bacterial endotoxins under Ceftizoxime for Injection. Where the label states that Ceftizoxime Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for Bacterial endotoxins under Ceftizoxime for Injection.

**Assay**—

**pH 3.6 Buffer**—Dissolve 1.42 g of citric acid monohydrate and 1.73 g of dibasic sodium phosphate in water to obtain 1000 mL of solution.

**pH 7.0 Buffer**—Dissolve 3.63 g of monobasic potassium phosphate and 10.73 g of dibasic sodium phosphate in water to obtain 1000 mL of solution.

**Mobile phase**—Prepare a mixture of pH 3.6 Buffer and acetonitrile (about 9:1). Filter through a filter (1  $\mu\text{m}$  or finer porosity), and degas. Adjust the composition, if necessary, to meet the performance requirements under Chromatographic system.

**Internal standard solution**—Dissolve 1.2 g of salicylic acid in 10 mL of methanol, and dilute with pH 7.0 Buffer to obtain 200 mL of solution.

**Standard preparation**—Dissolve a suitable quantity of USP Ceftizoxime RS, accurately weighed, in pH 7.0 Buffer to obtain a solution having a known concentration of about 1 mg of ceftizoxime ( $C_{13}H_{13}N_5O_5S_2$ ) per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of Internal standard solution, dilute with pH 7.0 Buffer to volume, and mix. This Standard preparation contains about 0.02 mg of ceftizoxime per mL.

**Assay preparation**—Using a suitable quantity of Ceftizoxime Sodium, accurately weighed, proceed as directed under Standard preparation.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.0-mm  $\times$  30-cm column that contains 5- to 10- $\mu\text{m}$  packing L1. The flow rate is about 2 mL per minute. Chromatograph the Standard preparation, and record the peak



responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 2000 theoretical plates; the tailing factor for the analyte peak is not more than 2, the resolution;  $R$ , between the analyte and internal standard peaks is not less than 4; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.6 for ceftizoxime and 1.0 for salicylic acid. Calculate the quantity, in  $\mu$ g, of ceftizoxime per mg of the Ceftizoxime Sodium taken by the formula:

$$1000(C / M)(R_U / R_S)$$

in which  $C$  is the concentration, in mg of ceftizoxime ( $C_{13}H_{13}N_5O_5S_2$ ) per mL, of the *Standard preparation*;  $M$  is the concentration, in mg per mL, of the *Assay preparation* based on the weight of Ceftizoxime Sodium taken and the extent of dilution; and  $R_U$  and  $R_S$  are the peak response ratios of the ceftizoxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ceftizoxime Injection

» Ceftizoxime Injection is a sterile solution of Ceftizoxime Sodium in a diluent containing one or more tonicity-adjusting agents in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of ceftizoxime ( $C_{13}H_{13}N_5O_5S_2$ ).

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

### USP Reference standards (11)—

USP Ceftizoxime RS  
USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftizoxime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.10 USP Endotoxin Unit per mg of ceftizoxime.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.5 and 8.0.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

### Assay—

*pH 3.6 Buffer, pH 7.0 Buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay* under *Ceftizoxime Sodium*.

*Assay preparation*—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the In-

jection, equivalent to about 40 mg of ceftizoxime, to a 100-mL volumetric flask, dilute with *pH 7.0 Buffer* to volume, and mix. Transfer 5.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Ceftizoxime Sodium*. Calculate the quantity, in mg, of ceftizoxime ( $C_{13}H_{13}N_5O_5S_2$ ) in each mL of the Injection taken by the formula:

$$2000(C / V)(R_U / R_S)$$

in which  $V$  is the volume, in mL, of Injection taken, and the other terms are as defined therein.

## Ceftizoxime for Injection

» Ceftizoxime for Injection contains an amount of Ceftizoxime Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of ceftizoxime ( $C_{13}H_{13}N_5O_5S_2$ ).

### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

### USP Reference standards (11)—

USP Ceftizoxime RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.10 USP Endotoxin Unit per mg of ceftizoxime.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It responds to the *Identification* tests and meets the requirements for *Crystallinity, pH, and Water* under *Ceftizoxime Sodium*. It meets also the requirements for *Uniformity of Dosage Units* (905) and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

### Assay—

*pH 3.6 Buffer, pH 7.0 Buffer, Mobile phase, Internal standard solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Ceftizoxime Sodium*.

*Standard preparation*—Dissolve a suitable quantity of USP Ceftizoxime RS, accurately weighed, in *pH 7.0 Buffer* to obtain a solution having a known concentration of about 1 mg of ceftizoxime ( $C_{13}H_{13}N_5O_5S_2$ ) per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix. This *Standard preparation* contains about 0.02 mg of ceftizoxime per mL.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Ceftizoxime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *pH 7.0 Buffer* to obtain a solution containing about 1 mg of ceftizoxime per mL. Transfer 2.0 mL of this solution to a



100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

**Assay preparation 2** (where the label states the quantity of ceftrizoxime in a given volume of constituted solution)—Constitute Ceftrizoxime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *pH 7.0 Buffer* to obtain a solution containing about 1 mg of ceftrizoxime per mL. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, dilute with *pH 7.0 Buffer* to volume, and mix.

**Procedure**—Proceed with Ceftrizoxime for Injection as directed for *Procedure* in the *Assay* under *Ceftrizoxime Sodium*. Calculate the quantity, in mg, of ceftrizoxime ( $C_{13}H_{13}N_5O_5S_2$ ) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(C)(R_U/R_S)$$

in which *L* is the labeled quantity, in mg of ceftrizoxime ( $C_{13}H_{13}N_5O_5S_2$ ), in the container, or in the volume of constituted solution taken, and *D* is the concentration, in mg of ceftrizoxime ( $C_{13}H_{13}N_5O_5S_2$ ) per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively; and the extent of dilution, *C* is the concentration, in mg of ceftrizoxime ( $C_{13}H_{13}N_5O_5S_2$ ) per mL, of the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of the ceftrizoxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ceftriaxone Injection

» Ceftriaxone Injection is a sterile solution of Ceftriaxone Sodium in a diluent containing one or more tonicity-adjusting agents in Water for Injection. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ).

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—

USP Ceftriaxone Sodium RS  
USP Ceftriaxone Sodium E-Isomer RS  
USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for ceftriaxone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of ceftriaxone.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 6.0 and 8.0.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

## Assay—

**pH 7.0 Buffer**—Dissolve 13.6 g of dibasic potassium phosphate and 4.0 g of monobasic potassium phosphate in water to obtain 1000 mL of solution. Adjust this solution with phosphoric acid or 10 N potassium hydroxide to a pH of  $7.0 \pm 0.1$ .

**pH 5.0 Buffer**—Dissolve 25.8 g of sodium citrate in 500 mL of water, adjust with citric acid solution (1 in 5) to a pH of  $5.0 \pm 0.1$ , and dilute with water to a volume of 1000 mL.

**Mobile phase**—Dissolve 3.2 g of tetraheptylammonium bromide in 400 mL of acetonitrile, add 44 mL of *pH 7.0 Buffer* and 4 mL of *pH 5.0 Buffer*, and add water to make 1000 mL. Pass through a membrane filter of 0.5- $\mu$ m or finer porosity, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ceftriaxone Sodium RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL. Use this solution promptly after preparation.

**Resolution solution**—Dissolve a suitable quantity of USP Ceftriaxone Sodium E-Isomer RS in *Standard preparation*, and dilute with *Mobile phase* to obtain a solution containing about 160  $\mu$ g of USP Ceftriaxone Sodium E-Isomer RS per mL and 160  $\mu$ g of USP Ceftriaxone Sodium RS per mL. Use this solution promptly after preparation.

**Assay preparation**—Allow 1 container of Injection to thaw, and mix. Transfer an accurately measured volume of the Injection, equivalent to about 40 mg of ceftriaxone, to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Use this solution promptly after preparation.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 270-nm detector and a 4.0-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*. The resolution, *R*, between the ceftriaxone E-isomer and ceftriaxone peaks is not less than 3. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*. The column efficiency determined from the analyte peak is not less than 1500 theoretical plates, the tailing factor for the analyte is not more than 2, and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ) in each mL of Injection taken by the formula:

$$200(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Ceftriaxone Sodium RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the ceftriaxone peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Ceftriaxone for Injection

### DEFINITION

Ceftriaxone for Injection contains an amount of Ceftriaxone Sodium equivalent to NLT 776  $\mu$ g/mg of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ), calculated on the anhydrous basis, and the equivalent of NLT 90.0% and NMT 115.0% of the labeled amount of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ).



**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****Change to read:**• **PROCEDURE**

- Protect solutions containing ceftriaxone sodium from light. (IRA 1-Aug-2016)

**Solution A:** 9 g/L of monobasic potassium phosphate in water

**Solution B:** 24 g/L of dibasic sodium phosphate, dodecahydrate in water

**Solution C:** 20 g/L of citric acid in water. Adjust with 10 N sodium hydroxide TS to a pH of 5.0 prior to dilution.

**Buffer:** Combine 389 mL of *Solution A* and 611 mL of *Solution B*. Adjust with 10 N sodium hydroxide TS or phosphoric acid to a pH of 7.0.

**Mobile phase:** Dissolve 2.0 g each of tetradecylammonium bromide and tetraheptylammonium bromide in a mixture of 440 mL of water, 55 mL of *Buffer*, 5.0 mL of *Solution C*, and 500 mL of acetonitrile.

**System suitability solution:** 50 µg/mL each of USP Ceftriaxone Sodium RS and USP Ceftriaxone Sodium *E*-isomer RS in *Mobile phase*

**Standard solution:** 0.3 mg/mL of USP Ceftriaxone Sodium RS in *Mobile phase*

**Sample solution 1:** Nominally 0.3 mg/mL of ceftriaxone from Ceftriaxone for Injection in *Mobile phase*

**Sample solution 2** (where it is represented as being in a single-dose container): Nominally 0.3 mg/mL of ceftriaxone in *Mobile phase* prepared as follows. Constitute Ceftriaxone for Injection in a volume of water corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents using a suitable hypodermic needle and syringe, and transfer to a suitable volumetric flask. Dilute with *Mobile phase* to volume.

**Sample solution 3** (where the label states the quantity of ceftriaxone in a given volume of constituted solution): Nominally 0.3 mg/mL of ceftriaxone in *Mobile phase* prepared as follows. Constitute Ceftriaxone for Injection in a volume of water corresponding to the volume of solvent specified in the labeling, and dilute with *Mobile phase* to final volume.

**Chromatographic system**

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for ceftriaxone and ceftriaxone *E*-isomer are 1.0 and 1.4, respectively.]

**Suitability requirements**

**Resolution:** NLT 3.0 between the ceftriaxone and ceftriaxone *E*-isomer peaks, *System suitability solution*

**Tailing factor:** NMT 2, *Standard solution*

**Relative standard deviation:** NMT 0.7%, *Standard solution*

**Analysis**

**Samples:** *Standard solution*, *Sample solution 1*, and *Sample solution 2* or *Sample solution 3*. (IRA 1-Aug-2016)

Calculate the quantity, in µg/mg, of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ) in the portion of Ceftriaxone for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response of ceftriaxone from *Sample solution 1*

$r_S$  = peak response of ceftriaxone from the *Standard solution*

$C_S$  = concentration of USP Ceftriaxone Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of ceftriaxone in *Sample solution 1* (mg/mL)

$P$  = potency of ceftriaxone in USP Ceftriaxone Sodium RS (µg/mg)

**Acceptance criteria:** NLT 776 µg/mg on the anhydrous basis

Calculate the percentage of the labeled amount of ceftriaxone ( $C_{18}H_{18}N_8O_7S_3$ ) in the portion of Ceftriaxone for Injection withdrawn from the container or in the portion of the constituted solution:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of ceftriaxone from *Sample solution 2* or *Sample solution 3*

$r_S$  = peak response of ceftriaxone from the *Standard solution*

$C_S$  = concentration of USP Ceftriaxone Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of ceftriaxone in *Sample solution 2* or *Sample solution 3* (mg/mL)

$P$  = potency of ceftriaxone in USP Ceftriaxone Sodium RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** 90.0%–115.0% of the labeled amount of ceftriaxone

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

**IMPURITIES****Change to read:**• **ORGANIC IMPURITIES**

- Protect solutions containing ceftriaxone sodium from light. (IRA 1-Aug-2016)

**Solution A, Solution B, Solution C, Buffer, Mobile phase, System suitability solution,** (IRA 1-Aug-2016) and **Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 3 µg/mL of USP Ceftriaxone Sodium RS in *Mobile phase*. (IRA 1-Aug-2016)

**Sample solution:** Nominally 0.3 mg/mL of ceftriaxone from Ceftriaxone for Injection in *Mobile phase*

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for ceftriaxone and ceftriaxone *E*-isomer are listed in *Table 1*.]

**Suitability requirements**

**Resolution:** NLT 3.0 between the ceftriaxone and ceftriaxone *E*-isomer

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Ceftriaxone for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*



- $r_s$  = peak response of ceftriaxone from the *Standard solution*  
 $C_s$  = concentration of USP Ceftriaxone Sodium RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of ceftriaxone in the *Sample solution* (mg/mL)  
 $P$  = potency of ceftriaxone in USP Ceftriaxone Sodium RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
**Acceptance criteria:** See Table 1. Disregard any peak below 0.1%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deacetylcefotaxime lactone <sup>a</sup>	0.20	0.5
7-Aminocephalosporanic acid <sup>b</sup>	0.34	— (IRA 1-Aug-2016)
Ceftriaxone triazine analog <sup>c</sup>	0.62	1.0
Ceftriaxone benzothiazolyl oxime <sup>d</sup>	0.72	0.2
Deacyl ceftriaxone <sup>e</sup>	0.78	1.0
Ceftriaxone	1.0	—
Ceftriaxone-3-ene isomer <sup>f</sup>	1.3	0.3
Ceftriaxone E-isomers <sup>g</sup>	1.4	1.0
Any individual unspecified impurity	—	0.2
Total impurities	—	5.0 (IRA 1-Aug-2016)

<sup>a</sup> (Z)-2-(2-Aminothiazol-4-yl)-N-[(5aR,6R)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]-2-(methoxyimino)acetamide.  
<sup>b</sup> Process impurities that are controlled in the drug substance are not to be reported, are not included in total impurities, and are listed here for information only. (IRA 1-Aug-2016)  
<sup>c</sup> 3-Mercapto-2-methyl-1,2-dihydro-1,2,4-triazine-5,6-dione.  
<sup>d</sup> (Z)-5-Benzothiazol-2-yl-2-(2-aminothiazol-4-yl)-2-(methoxyimino)thioacetate.  
<sup>e</sup> (6R,7R)-7-Amino-3-[[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.  
<sup>f</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.  
<sup>g</sup> (6R,7R)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

### SPECIFIC TESTS

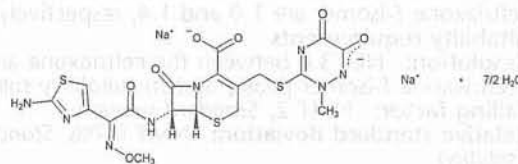
- CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions*.
- BACTERIAL ENDOTOXINS TEST** (85): NMT 0.20 USP Endotoxin Units/mg of ceftriaxone
- STERILITY TESTS** (71), *Test for Sterility of the Product to Be Examined, Membrane Filtration:* It meets the requirements.
- PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections
- CRYSTALLINITY** (695): Meets the requirements
- PH** (791)  
 Sample solution: 100 mg/mL  
 Acceptance criteria: 6.0–8.0
- WATER DETERMINATION** (921), *Method I:* 8.0%–11.0%
- OTHER REQUIREMENTS:** It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*.

### ADDITIONAL REQUIREMENTS

#### Change to read:

- PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution*, (CN 1-May-2017) and protected from light. (IRA 1-Aug-2016)
- USP REFERENCE STANDARDS** (11)  
 USP Ceftriaxone Sodium RS  
 USP Ceftriaxone Sodium E-Isomer RS  
 (6R,7R)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, disodium salt.  
 $\text{C}_{18}\text{H}_{16}\text{N}_8\text{Na}_2\text{O}_7\text{S}_3$  598.53  
 USP Endotoxin RS

### Ceftriaxone Sodium



- $\text{C}_{18}\text{H}_{16}\text{N}_8\text{Na}_2\text{O}_7\text{S}_3 \cdot 3\frac{1}{2}\text{H}_2\text{O}$  661.60  
 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[[(2-amino-4-thiazolyl)(methoxyimino)acetyl]amino]-8-oxo-3-[[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-1,2,4-triazin-3-yl)thio]methyl]-disodium salt, [6R-[6 $\alpha$ ,7 $\beta$ (Z)]]-, hydrate, (2:7);  
 (6R,7R)-7-[2-(2-Amino-4-thiazolyl)glyoxylamido]-8-oxo-3-[[[(1,2,5,6-tetrahydro-2-methyl-5,6-dioxo-*as*-triazin-3-yl)thio]methyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7 $\alpha$ -(Z)-(O-methyloxime), disodium salt, hemisepatahydrate [104376-79-6].  
 Anhydrous 598.56

### DEFINITION

Ceftriaxone Sodium contains the equivalent of NLT 795  $\mu\text{g}/\text{mg}$  of ceftriaxone ( $\text{C}_{18}\text{H}_{16}\text{N}_8\text{O}_7\text{S}_3$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- C. IDENTIFICATION TESTS—GENERAL** (191), *Sodium*

### ASSAY

#### Change to read:

#### PROCEDURE

- Protect solutions containing ceftriaxone sodium from light. (IRA 1-Aug-2016)  
**Solution A:** 9 g/L of monobasic potassium phosphate in water  
**Solution B:** 24 g/L of dibasic sodium phosphate, dodecahydrate in water  
**Solution C:** 20 g/L of citric acid in water. Adjust with 10 N sodium hydroxide to a pH of 5.0 prior to final dilution.



**Buffer:** Combine 389 mL of *Solution A* and 611 mL of *Solution B*. Adjust with 10 N sodium hydroxide TS or phosphoric acid to a pH of 7.0.

**Mobile phase:** Dissolve 2.0 g each of tetradecylammonium bromide and tetraheptylammonium bromide in a mixture of 440 mL of water, 55 mL of *Buffer*, 5.0 mL of *Solution C*, and 500 mL of acetonitrile.

**System suitability solution:** 50 µg/mL of USP Ceftriaxone Sodium RS and 50 µg/mL of USP Ceftriaxone Sodium *E*-isomer RS in *Mobile phase*

**Standard solution:** 0.3 mg/mL of USP Ceftriaxone Sodium RS in *Mobile phase*

**Sample solution:** 0.3 mg/mL of Ceftriaxone Sodium in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for ceftriaxone and ceftriaxone *E*-isomer are 1.0 and 1.4, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between the ceftriaxone and ceftriaxone *E*-isomer peaks, *System suitability solution*

**Tailing factor:** NMT 2, *Standard solution*

**Relative standard deviation:** NMT 0.7%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of ceftriaxone (C<sub>18</sub>H<sub>18</sub>N<sub>8</sub>O<sub>7</sub>S<sub>3</sub>) in the portion of Ceftriaxone Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response of ceftriaxone from the *Sample solution*

$r_S$  = peak response of ceftriaxone from the *Standard solution*

$C_S$  = concentration of USP Ceftriaxone Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Ceftriaxone Sodium in the *Sample solution* (mg/mL)

$P$  = potency of ceftriaxone in USP Ceftriaxone Sodium RS (µg/mg)

**Acceptance criteria:** NLT 795 µg/mg on the anhydrous basis

#### IMPURITIES

##### Change to read:

##### • ORGANIC IMPURITIES

• Protect solutions containing ceftriaxone sodium from light. (IRA 1-Aug-2016)

**Solution A, Solution B, Solution C, Buffer, Mobile phase, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution:** 3 µg/mL of USP Ceftriaxone Sodium RS in *Mobile phase*

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for ceftriaxone and ceftriaxone *E*-isomer are listed in *Table 1*.]

#### Suitability requirements

**Resolution:** NLT 3.0 between the ceftriaxone *E*-isomer and ceftriaxone peaks, *System suitability solution*

**Signal-to-noise ratio:** NLT 10, *Standard solution*

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

Calculate the percentage of each individual impurity in the portion of Ceftriaxone Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of ceftriaxone from the *Standard solution*

$C_S$  = concentration of USP Ceftriaxone Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Ceftriaxone Sodium in the *Sample solution* (mg/mL)

$P$  = potency of ceftriaxone in USP Ceftriaxone Sodium RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** See *Table 1*. Disregard any peak below 0.1%.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Deacetylcefotaxime lactone <sup>a</sup>	0.20	0.5
7-Aminocephalosporanic acid <sup>b,c</sup> (if present)	0.34	0.5
Ceftriaxone triazine analog <sup>d</sup>	0.62	1.0
Ceftriaxone benzothiazolyloxime <sup>e</sup>	0.72	0.2
Deacyl ceftriaxone <sup>f</sup>	0.78	0.5
Ceftriaxone	1.0	—
Ceftriaxone 3-ene isomer <sup>g</sup>	1.3	0.3
Ceftriaxone <i>E</i> -isomer <sup>h</sup>	1.4	0.5
Any individual unspecified impurity	—	0.2
Total impurities	—	2.5

<sup>a</sup> (Z)-2-(2-Aminothiazol-4-yl)-N-[(5aR,6R)-1,7-dioxo-1,3,4,5a,6,7-hexahydroazeto[2,1-b]furo[3,4-d][1,3]thiazin-6-yl]-2-(methoxyimino)acetamide.

<sup>b</sup> 7-ACA; (6R,7R)-3-(Acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>c</sup> To be reported if present in the impurity profile.

<sup>d</sup> 3-Mercapto-2-methyl-1,2-dihydro-1,2,4-triazine-5,6-dione.

<sup>e</sup> (Z)-5-Benzothiazol-2-yl 2-(2-aminothiazol-4-yl)-2-(methoxyimino)thioacetate.

<sup>f</sup> (6R,7R)-7-Amino-3-[[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

<sup>g</sup> (6R,7R)-7-[(Z)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-3-ene-2-carboxylic acid.

<sup>h</sup> (6R,7R)-7-[(E)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid.

#### SPECIFIC TESTS

• **CRYSTALLINITY** (695): Meets the requirements

• **PH** (791)

**Sample solution:** 100 mg/mL

**Acceptance criteria:** 6.0–8.0

• **WATER DETERMINATION** (921), *Method I*: 8.0%–11.0%

• **STERILITY TESTS** (71), *Test for Sterility of the Product to Be Examined, Membrane Filtration*: Where the label states that it is sterile, it meets the requirements.

• **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.20 USP Endotoxin Units/mg of ceftriaxone.



**ADDITIONAL REQUIREMENTS****Change to read:**

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. (IRA 1-Aug-2016)
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS (11)**
  - USP Ceftriaxone Sodium RS
  - USP Ceftriaxone Sodium *E*-Isomer RS
  - (6*R*,7*R*)-7-[(*E*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[[[(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)thio]methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, disodium salt.
  - $C_{18}H_{16}N_8Na_2O_7S_3$  598.53
  - USP Endotoxin RS

**Cefuroxime Injection**

» Cefuroxime Injection is a sterile isoosmotic solution of Cefuroxime Sodium in Water for Injection. It contains one or more suitable buffers and a tonicity-adjusting agent. It contains not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ).

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards (11)**—

USP Cefuroxime Sodium RS  
USP Endotoxin RS

**Identification**—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefuroxime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.10 USP Endotoxin Unit per mg of cefuroxime.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 5.0 and 7.5.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905).

**Assay**—

pH 3.4 Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system—Prepare as directed in the *Assay under Cefuroxime Sodium*.

*Assay preparation*—Allow a container of Injection to thaw, and mix the solution. Transfer an accurately measured volume of the Injection, equivalent to about 50 mg of cefuroxime, to a 50-mL volumetric flask, dilute with water to volume, and mix. Immediately transfer 5.0 mL of this solution

to a second 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay under Cefuroxime Sodium*. Calculate the quantity, in mg, of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) in each mL of the Injection taken by the formula:

$$1000(C/V)(R_U/R_S)$$

in which *V* is the volume, in mL, of Injection taken, and the other terms are as defined therein.

**Cefuroxime for Injection**

» Cefuroxime for Injection contains an amount of Cefuroxime Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ).

**Change to read:**

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for constitution* (CN 1-May-2017).

**USP Reference standards (11)**—

USP Cefuroxime Sodium RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, the constituted solution for intravenous administration prepared from Cefuroxime for Injection meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.10 USP Endotoxin Unit per mg of cefuroxime.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**Uniformity of dosage units** (905): meets the requirements.

*Procedure for content uniformity*—Perform the *Assay* on individual containers using *Assay preparation 1* or *Assay preparation 2*, or both, as appropriate.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements of the tests for *Identification*, *pH*, and *Water* under *Cefuroxime Sodium*. It meets also the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*.

**Assay**—

pH 3.4 Acetate buffer, Mobile phase, Internal standard solution, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay under Cefuroxime Sodium*.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Cefuroxime for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with water to obtain a solution containing about 1 mg of cefuroxime per mL. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

*Assay preparation 2* (where the label states the quantity of cefuroxime in a given volume of constituted solution or suspension)—Constitute Cefuroxime for Injection in a volume of water, accurately measured, corresponding to the



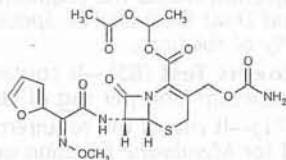
volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution or suspension quantitatively with water to obtain a solution containing about 1 mg of cefuroxime per mL. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Cefuroxime Sodium*. Calculate the quantity, in mg, of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) withdrawn from the container, or in the portion of constituted solution or suspension taken by the formula:

$$(L / D)(C)(R_U / R_S)$$

in which  $L$  is the labeled quantity, in mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ), in the container, or in the volume of constituted solution or suspension taken;  $D$  is the concentration, in mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution or suspension taken, respectively, and the extent of dilution;  $C$  is the concentration, in mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mL, of the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of cefuroxime to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively. Where the test for *Uniformity of dosage units* has been performed using the *Procedure for content uniformity*, use the average of these determinations as the *Assay value*.

## Cefuroxime Axetil



$C_{20}H_{22}N_4O_{10}S$  510.47

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[[(aminocarbonyl)oxy]methyl]-7-[[2-furyl] (methoxyimino)acetyl]amino]-8-oxo-, 1-(acetyloxy)ethyl ester, [6R-[6 $\alpha$ ,7 $\beta$ (Z)]]-

(RS)-1-Hydroxyethyl (6R,7R)-7-[2-(2-furyl)glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, 7 $\alpha$ -(Z)-(O-methyloxime), 1-acetate 3-carbamate [64544-07-6].

» Cefuroxime Axetil is a mixture of the diastereoisomers of cefuroxime axetil ( $C_{20}H_{22}N_4O_{10}S$ ). It contains the equivalent of not less than 745  $\mu$ g and not more than 875  $\mu$ g of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate whether it is amorphous or crystalline.

**USP Reference standards** (11)—

USP Cefuroxime Axetil RS

USP Cefuroxime Axetil Delta-3 Isomers RS

**Identification**, *Infrared Absorption* (197K).

**Crystallinity** (695)—Particles that do not show birefringence or exhibit extinction positions are amorphous, and particles that show birefringence and exhibit extinction positions are crystalline.

**Water**, *Method I* (921): not more than 1.5%.

**Diastereoisomer ratio**—

0.2 M Monobasic ammonium phosphate, Mobile phase, Internal standard solution, Resolution solution, Standard preparation, Assay preparation, and Chromatographic system—Prepare as directed in the Assay.

**Procedure**—Proceed as directed for *Procedure* in the Assay. Calculate the ratio of cefuroxime axetil diastereoisomer A to the sum of the cefuroxime axetil diastereoisomers A and B taken by the formula:

$$r_A / (r_A + r_B)$$

in which  $r_A$  and  $r_B$  are the peak responses of the cefuroxime axetil diastereoisomers A and B, respectively: between 0.48 and 0.55 is obtained.

**Assay**—

0.2 M Monobasic ammonium phosphate—Dissolve 23.0 g of monobasic ammonium phosphate in water to obtain 1000 mL of solution.

**Mobile phase**—Prepare a suitable filtered and degassed mixture of 0.2 M Monobasic ammonium phosphate and methanol (620: 380). Make adjustments if necessary (see System Suitability under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of acetanilide in methanol containing 5.4 mg per mL.

**Resolution solution**—In a 50-mL volumetric flask, mix 10.0 mL of a solution of USP Cefuroxime Axetil RS in methanol containing 1.2 mg per mL, 5.0 mL of Internal standard solution, and 3.8 mL of a solution of USP Cefuroxime Axetil Delta-3 Isomers RS in methanol containing 0.16 mg per mL. Dilute with 0.2 M Monobasic ammonium phosphate to volume, and mix.

**Standard preparation**—Transfer about 30 mg of USP Cefuroxime Axetil RS, accurately weighed, to a 25-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Promptly transfer 10.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of Internal standard solution and 3.8 mL of methanol, dilute with 0.2 M Monobasic ammonium phosphate to volume, and mix. [NOTE—Use this Standard preparation promptly, or refrigerate and use on the day prepared.]

**Assay preparation**—Transfer about 30 mg of Cefuroxime Axetil to a 25-mL volumetric flask, dissolve in methanol, dilute with methanol to volume, and mix. Promptly transfer 10.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of Internal standard solution and 3.8 mL of methanol, dilute with 0.2 M Monobasic ammonium phosphate to volume, and mix. [NOTE—Use this Assay preparation promptly, or refrigerate and use on the day prepared.]

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 278-nm detector and a 4.6-mm  $\times$  25-cm column containing 5- $\mu$ m packing L13. The flow rate is about 1.5 mL per minute. Chromatograph the Resolution solution, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for acetanilide, 0.8 for cefuroxime axetil diastereoisomer B, 0.9 for cefuroxime axetil diastereoisomer A, and 1.0 for cefuroxime axetil delta-3 isomers; the resolution,  $R$ , between cefuroxime axetil diastereoisomer A and B is not less than 1.5; and the resolution,  $R$ , between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers is not less than 1.5. Chromatograph the Standard preparation, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 3000 theoretical plates when measured using the cefuroxime axetil diastereoisomer A peak; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the Standard preparation and the Assay preparation into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quan-



tity, in  $\mu\text{g}$ , of cefuroxime ( $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$ ) in each mg of Cefuroxime Axetil taken by the formula:

$$(W_s/W_u)(P_s/100)(100 - K)(R_u/R_s)$$

in which  $W_s$  is the weight, in mg, of USP Cefuroxime Axetil RS taken to prepare the *Standard preparation*;  $W_u$  is the weight, in mg, of Cefuroxime Axetil taken to prepare the *Assay preparation*;  $P_s$  is the designated cefuroxime ( $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$ ) content, in  $\mu\text{g}$  per mg, of anhydrous USP Cefuroxime Axetil RS;  $K$  is the percentage water content of USP Cefuroxime Axetil RS; and  $R_u$  and  $R_s$  are the ratios of the sum of the peak responses of the cefuroxime axetil diastereoisomers A and B to the peak response of the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cefuroxime Axetil for Oral Suspension

### DEFINITION

Cefuroxime Axetil for Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of cefuroxime ( $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$ ).

### IDENTIFICATION

- The retention times of the major peaks for cefuroxime axetil diastereoisomers A and B of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Solution A:** 23 g/L of monobasic ammonium phosphate in water

**Mobile phase:** Methanol and *Solution A* (19:31)

**System suitability stock solution A:** 1.2 mg/mL of USP Cefuroxime Axetil RS in methanol

**System suitability stock solution B:** 0.16 mg/mL of USP Cefuroxime Axetil Delta-3 Isomers RS in methanol

**System suitability solution:** Transfer 10.0 mL of *System suitability stock solution A* to a 50-mL volumetric flask. Add 5.0 mL of methanol and 3.8 mL of *System suitability stock solution B*. Dilute with *Solution A* to volume.

**Standard stock solution:** 1.2 mg/mL of USP Cefuroxime Axetil RS in methanol. [NOTE—Use this solution promptly.]

**Standard solution:** Transfer 10.0 mL of *Standard stock solution* to a 50-mL volumetric flask, add 8.8 mL of methanol, and dilute with *Solution A* to volume.

[NOTE—Use this *Standard solution* promptly, or refrigerate and use on the day prepared.]

**Sample stock solution:** Equivalent to 2.5 mg/mL of cefuroxime, from constituted Oral Suspension, in methanol. Pass through a suitable filter. [NOTE—Constitute as directed on the label. To a suitable aliquot, freshly prepared and free of bubbles, add a suitable volume of methanol, shake by mechanical means for 10 min, dilute to volume with methanol, and mix.]

**Sample solution:** Transfer 5.0 mL of the filtered *Sample stock solution* to a 50-mL volumetric flask. Add 13.8 mL of methanol, and dilute with *Solution A* to volume.

[NOTE—Protect the *Sample solution* from light and use promptly, or refrigerate and use on the day prepared.]

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 278 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu\text{m}$  packing L13

**Flow rate:** 1.5 mL/min

**Injection size:** 10  $\mu\text{L}$

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for acetanilide, cefuroxime axetil diastereoisomer B, cefuroxime axetil diastereoisomer A, and cefuroxime axetil delta-3 isomers are 0.4, 0.8, 0.9, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between cefuroxime axetil diastereoisomer A and B; NLT 1.5 between cefuroxime axetil diastereoisomer A and cefuroxime axetil delta-3 isomers, *System suitability solution*

**Column efficiency:** NLT 3000 theoretical plates when measured using the cefuroxime axetil diastereoisomer A peak, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$  in the Cefuroxime Axetil for Oral Suspension taken:

$$\text{Result} = (R_u/R_s) \times (C_s/C_u) \times P \times F \times [1 - (K/100)] \times 100$$

$R_u$  = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Sample solution*

$R_s$  = sum of the peak responses of cefuroxime axetil diastereoisomers A and B from the *Standard solution*

$C_s$  = concentration of the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of cefuroxime axetil in the *Sample solution* (mg/mL)

$P$  = potency of cefuroxime in anhydrous USP Cefuroxime Axetil RS ( $\mu\text{g}/\text{mg}$ )

$F$  = unit conversion factor, 0.001 mg/ $\mu\text{g}$

$K$  = water content of USP Cefuroxime Axetil RS (%)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION <711>

**Medium:** 0.07 M of pH 7.0 phosphate buffer (dissolve 3.7 mg/mL of monobasic sodium phosphate and 5.7 mg/mL of anhydrous dibasic sodium phosphate in water); 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Analysis:** Test 5.0 mL of constituted Cefuroxime Axetil for Oral Suspension equivalent to 125 or 250 mg of cefuroxime. Determine the amount of cefuroxime equivalent dissolved by using UV absorption at the wavelength of maximum absorbance at 280 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Cefuroxime Axetil RS in the same *Medium*.

**Tolerances:** NLT 60% (Q) of the labeled amount of  $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$  is dissolved.

#### UNIFORMITY OF DOSAGE UNITS <905>

**For solid packaged in single-unit containers:** Constitute Cefuroxime Axetil for Oral Suspension as directed in the labeling. Mix, and allow the container to drain into a beaker for 5 s. Withdraw and assay 5.0 mL of the Oral Suspension from the beaker, or the total amount if it is less than 5 mL. It meets the requirements.



• **DELIVERABLE VOLUME** (698)

For solid packaged in multiple-unit containers: Constitute Cefuroxime Axetil for Oral Suspension as directed in the labeling. It meets the requirements.

**SPECIFIC TESTS**

- **PH** (791): 3.5–7.0, in the solution constituted as directed in the labeling
- **WATER DETERMINATION, Method I** (921): NMT 6.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
USP Cefuroxime Axetil RS  
USP Cefuroxime Axetil Delta-3 Isomers RS

## Cefuroxime Axetil Tablets

» Cefuroxime Axetil Tablets contain the equivalent of not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ).

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—The labeling indicates whether the Tablets contain amorphous or crystalline Cefuroxime Axetil. If Tablets contain a mixture of amorphous and crystalline Cefuroxime Axetil, label to indicate the percentage of each contained therein. When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

**USP Reference standards** (11)—

USP Cefuroxime Axetil RS  
USP Cefuroxime Axetil Delta-3 Isomers RS

**Identification**—The retention times of the major peaks for cefuroxime axetil diastereoisomers A and B in the chromatogram of the *Assay preparation* correspond to those in the chromatogram of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Dissolution** (711)—

TEST 1—

*Medium:* 0.07 N hydrochloric acid; 900 mL.

*Apparatus 2:* 55 rpm.

*Times:* 15 and 45 minutes.

*Procedure*—Determine the amount of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) dissolved by employing UV absorption at the wavelength of maximum absorbance at about 278 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Cefuroxime Axetil RS, equivalent to about 0.01 to 0.02 mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mL, in the same *Medium*.

**Tolerances**—Not less than 60% (Q) of the labeled amount of  $C_{16}H_{16}N_4O_8S$  is dissolved in 15 minutes, and not less than 75% (Q) is dissolved in 45 minutes; except that where Tablets are labeled to contain the equivalent of 500 mg of cefuroxime, not less than 50% (Q) of the labeled amount of  $C_{16}H_{16}N_4O_8S$  is dissolved in 15 minutes, and not less than 70% (Q) is dissolved in 45 minutes.

TEST 2—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

*Apparatus 2:* 100 rpm.

*Medium, Times, and Procedure*—Proceed as directed under *Test 1*.

**Tolerances**—Not less than 60% (Q) of the labeled amount of  $C_{16}H_{16}N_4O_8S$  is dissolved in 15 minutes, and not less than

75% (Q) of the labeled amount of  $C_{16}H_{16}N_4O_8S$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water Determination, Method I** (921): not more than 6.0%.

**Assay**—

0.2 M Monobasic ammonium phosphate, Mobile phase, Internal standard solution, Resolution solution, Standard preparation, and Chromatographic system—Proceed as directed in the *Assay* under Cefuroxime Axetil.

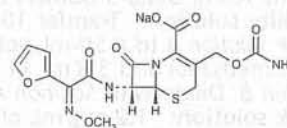
*Assay preparation*—Finely powder not fewer than 10 Tablets, accurately counted. Transfer the powder, with the aid of methanol, to a volumetric flask of such capacity that when filled to volume, the solution will contain the equivalent of about 2 mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mL. Add methanol to fill the volumetric flask to about half of its capacity, and shake by mechanical means for about 10 minutes. Dilute with methanol to volume, and mix. Filter a portion of this stock mixture, and transfer 5.0 mL of the filtrate to a 50-mL volumetric flask. Add 5.0 mL of *Internal standard solution* and 8.8 mL of methanol, dilute with 0.2 M Monobasic ammonium phosphate to volume, and mix. [NOTE—Use this *Assay preparation* promptly, or refrigerate and use on the day prepared.]

*Procedure*—Proceed as directed in the *Assay* under Cefuroxime Axetil. Calculate the quantity, in mg, of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) in each Tablet taken by the formula:

$$(V/12,500N)(P_5W_5/100)(100 - K)(R_U / R_S)$$

in which *V* is the volume, in mL, of the volumetric flask used to prepare the stock mixture; *N* is the number of Tablets taken; and the other terms are as defined therein.

## Cefuroxime Sodium



$C_{16}H_{15}N_4NaO_8S$  446.37

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[[[aminocarbonyl]oxy]methyl]-7-[[2-furanyl(methoxymino)acetyl]amino]-8-oxo-, monosodium salt [6R-[6 $\alpha$ ,7 $\beta$ (Z)]]-

Sodium (6R,7R)-7-[2-(2-furyl)glyoxylamido]-3-(hydroxymethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate, 7<sup>2</sup>-(Z)-(O-methyloxime), carbamate (ester) [56238-63-2].

» Cefuroxime Sodium contains the equivalent of not less than 855  $\mu$ g and not more than 1000  $\mu$ g of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ), calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cefuroxime Sodium RS  
USP Endotoxin RS



**Identification—**

**A:** The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for cefuroxime, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained as directed in the *Assay*.

**B:** It responds to the tests for *Sodium* (191).

**pH** (791): between 6.0 and 8.5, in a solution (1 in 10).

**Water Determination, Method I** (921): not more than 3.5%.

**Other requirements—**Where the label states that Cefuroxime Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cefuroxime for Injection*. Where the label states that Cefuroxime Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cefuroxime for Injection*.

**Assay—**

**pH 3.4 acetate buffer—**Transfer 50 mL of 0.1 M sodium acetate to a 1000-mL volumetric flask, dilute with 0.1 N acetic acid to volume, and mix.

**Mobile phase—**Prepare a suitable mixture of pH 3.4 acetate buffer and acetonitrile (about 10:1). Filter through a membrane filter (1 µm or finer porosity), and degas.

**Internal standard solution—**Prepare a solution of orcinol in water containing 1.5 mg per mL.

**Standard preparation—**Dissolve a suitable quantity of USP Cefuroxime Sodium RS, accurately weighed, in water to obtain a solution having a known concentration of about 1 mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mL. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix. This *Standard preparation* contains about 0.05 mg of cefuroxime per mL.

**Assay preparation—**Using a suitable quantity of Cefuroxime Sodium, accurately weighed, proceed as directed in the first sentence under *Standard preparation*. Immediately transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, add 20.0 mL of *Internal standard solution*, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains 5-µm packing L15. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 1300 theoretical plates; the tailing factor for the analyte peak is not more than 2.0; the resolution, *R*, between the analyte and internal standard peaks is not less than 3.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure—**Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for cefuroxime and 1.0 for orcinol. Calculate the quantity, in µg, of cefuroxime per mg of the Cefuroxime Sodium taken by the formula:

$$1000(C/M)(R_U / R_S)$$

in which *C* is the concentration, in mg of cefuroxime ( $C_{16}H_{16}N_4O_8S$ ) per mL, in the *Standard preparation*; *M* is the concentration, in mg per mL, in the *Assay preparation* based on the weight of Cefuroxime Sodium taken and the extent of dilution; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of the cefuroxime peak to the internal standard peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

**Celecoxib**

$C_{17}H_{14}F_3N_3O_2S$  381.4  
4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;  
*p*-[5-*p*-Tolyl-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide [169590-42-5].

**DEFINITION**

Celecoxib contains NLT 98.0% and NMT 102.0% of  $C_{17}H_{14}F_3N_3O_2S$ , calculated on the anhydrous basis.

**IDENTIFICATION**

- A. INFRARED ABSORPTION** (197): [NOTE—Methods (197A), (197K), or (197M) under *Infrared Absorption* may be used.]  
[NOTE—If the spectra obtained show differences, dissolve the substance to be examined and the Reference Standard separately in isopropyl alcohol, evaporate to dryness, and record the new spectra.]
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE**

**Buffer:** 2.7 g/L of monobasic potassium phosphate adjusted with phosphoric acid to a pH of  $3.0 \pm 0.2$

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (3:1:6)

**Diluent:** Methanol and water (3:1)

**System suitability solution:** 0.5 mg/mL of USP

Celecoxib RS and 2.4 µg/mL each of USP Celecoxib Related Compound A RS and USP Celecoxib Related Compound B RS in *Diluent*

**Standard solution:** 0.5 mg/mL of USP Celecoxib RS in *Diluent*

**Sample solution:** 0.5 mg/mL of Celecoxib in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L11

**Column temperature:** 60°

**Flow rate:** 1.5 mL/min

**Injection size:** 25 µL

**Run time:** About 1.5 times the celecoxib peak elution

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.8 between celecoxib related compound A and celecoxib and NLT 1.8 between celecoxib and celecoxib related compound B, *System suitability solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{17}H_{14}F_3N_3O_2S$  in the portion of Celecoxib taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of the *Standard solution* (mg/mL)

*C<sub>U</sub>* = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis



## IMPURITIES

### Inorganic Impurities

#### Delete the following:

- **HEAVY METALS:** NMT 20 ppm  
**Diluent:** Acetone and water (17:3)  
**Standard solution:** Dilute 1.0 mL of *Standard Lead Solution*, prepared as directed under *Heavy Metals* (231), *Special Reagents*, with *Diluent* to 20 mL.  
**Sample solution:** Dissolve 0.50 g of Celecoxib in 20 mL of *Diluent*.

**Blank solution:** 20 mL of *Diluent*

#### Analysis

**Samples:** *Standard solution*, *Blank solution*, and *Sample solution*

To each solution, add 2 mL of pH 3.5 *Acetate Buffer*, prepared as directed under *Heavy Metals* (231), *Method 1*. Mix, and add to each solution 1.2 mL of thioacetamide–glycerin base TS. Mix immediately, and allow to stand for 2 min. Pass the solutions through a filter of 0.45-μm pore size. Compare the spots on the filters obtained from each of the solutions.

**Acceptance criteria:** The brownish-black color of the spot resulting from the *Sample solution* is not more intense than that of the spot resulting from the *Standard solution*. The test is invalid if the *Standard solution* does not show a brownish-black color compared to the *Blank solution*. • (Official 1-Jan-2018)

- **RESIDUE ON IGNITION** (281): NMT 0.2%, using a platinum crucible

### Organic Impurities

#### • PROCEDURE

**Buffer, Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.5 μg/mL of USP Celecoxib RS in *Diluent*

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.8 between celecoxib related compound A and celecoxib and NLT 1.8 between celecoxib and celecoxib related compound B, *System suitability solution*

**Signal-to-noise ratio:** NLT 20, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Celecoxib taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response for each impurity in the *Sample solution*  
 $r_s$  = peak response of celecoxib in the *Standard solution*  
 $C_s$  = concentration of celecoxib in the *Standard solution* (mg/mL)  
 $C_u$  = concentration of Celecoxib in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Individual impurities:** See *Table 1*.

[NOTE—Disregard any impurity peak less than 0.05%.]

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Celecoxib related compound A <sup>a</sup>	0.9	0.4
Celecoxib	1.0	—
Celecoxib related compound B <sup>b</sup>	1.1	0.10
Individual unspecified impurity	—	0.10
Total impurities	—	0.5

<sup>a</sup> 4-[5-(3-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide.

<sup>b</sup> 4-[3-(4-Methylphenyl)-5-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide.

### SPECIFIC TESTS

- **WATER DETERMINATION, Method I** (921): NMT 0.5%, using a 400-mg sample

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and moisture. Store at room temperature.

- **USP REFERENCE STANDARDS** (11)

USP Celecoxib RS

*p*-[5-*p*-Tolyl-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide.

C<sub>17</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>S 381.4

USP Celecoxib Related Compound A RS

4-[5-(3-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide.

C<sub>17</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>S 381.4

USP Celecoxib Related Compound B RS

4-[3-(4-Methylphenyl)-5-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide.

C<sub>17</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>S 381.4

## Oxidized Cellulose

### DEFINITION

Oxidized Cellulose contains NLT 16.0% and NMT 24.0% of carboxyl groups (COOH), calculated on the dried basis. It is sterile.

### IDENTIFICATION

#### • A.

**Sample solution:** 200 mg in 10 mL of 0.25 N sodium hydroxide

**Analysis 1:** Shake the *Sample solution* for 1 min. Add 10 mL of water, and shake.

**Acceptance criteria 1:** The *Sample solution* shows no more than a slight haze and is substantially free from fibers and foreign particles.

**Analysis 2:** Allow the resulting solution to stand for 10 min.

**Acceptance criteria 2:** Any swollen fibers initially present are no longer visible.

**Analysis 3:** Acidify the resulting solution with 3 N hydrochloric acid.

**Acceptance criteria 3:** A flocculent white precipitate is formed.

### ASSAY

#### • PROCEDURE

**Solution A:** 20 mg/mL of calcium acetate

**Sample:** 500 mg, previously dried under vacuum over phosphorus pentoxide for 18 h



Blank: 50.0 mL of *Solution A*

#### Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N sodium hydroxide VS

Endpoint detection: Visual

**Analysis:** Place the *Sample* in a 125-mL conical flask. Add 50.0 mL of *Solution A*, swirl until the sample is completely covered, allow the mixture to stand for 30 min, then add phenolphthalein TS. Titrate the solution with *Titrant*. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 4.502 mg of carboxyl groups (COOH).

**Acceptance criteria:** 16.0%–24.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.15%

- **LIMIT OF NITROGEN**

*Solution A:* 40 mg/mL of boric acid

*Solution B:* Methyl red TS and bromocresol green TS (1:4)

*Sample:* 1 g, previously dried under vacuum over phosphorus pentoxide for 18 h

#### Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.02 N sulfuric acid VS

Endpoint detection: Visual

**Analysis:** Place a 125-mL conical flask, containing 30 mL of *Solution A* and 6 drops of *Solution B*, beneath the condenser of the distillation apparatus so that the tip of the condenser is well below the surface of the resulting solution. To a 500-mL Kjeldahl flask, add the *Sample*, and add 1 g of Devarda's alloy, 100 mL of recently boiled water, a small lump of paraffin, and 100 mL of 1 N sodium hydroxide. Connect the Kjeldahl flask to the condenser by a suitable trap bulb. Heat the mixture in the flask until 45–50 mL of distillate has collected in the receiver. Rinse the condenser, and titrate the resulting solution with *Titrant* to a pale pink endpoint. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 0.2801 mg of nitrogen.

**Acceptance criteria:** NMT 0.5%

- **LIMIT OF FORMALDEHYDE**

*Solution A:* Formaldehyde in water (1 in 40,000)

*Standard:* 0.50 mL of *Solution A*

*Sample:* 500 mg

#### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: Vis

Analytical wavelength: 570 nm

Blank: Mixture of 0.5 mL of water and 10 mL of chromotropic acid TS

**Analysis:** Transfer the *Sample* to a 500-mL iodine flask. Add 250 mL of water, and allow to stand for NLT 2 h with intermittent shaking. Pipet 0.50 mL each of the supernatant from the resulting solution and the *Standard* into two separate glass-stoppered test tubes. To each test tube add 10 mL of chromotropic acid TS. Stopper the tubes loosely, and heat in a boiling water bath for 30 min. Cool, and determine the absorbance of each solution against the *Blank*.

**Acceptance criteria:** 0.5%; the absorbance of the *Sample* is NMT the *Standard*.

#### SPECIFIC TESTS

- **STERILITY TESTS** (71)

*Sample:* 250 mg

**Analysis:** Proceed as directed in the chapter, adding 0.5 mL of 0.1 N sodium hydroxide to the portions of media used.

**Acceptance criteria:** Meets the requirements

- **LOSS ON DRYING** (731)

**Analysis:** Dry under vacuum over phosphorus pentoxide for 18 h.

**Acceptance criteria:** NMT 15.0%

#### ADDITIONAL REQUIREMENTS

##### Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017), protected from direct sunlight. Store in a cold place.
- **LABELING:** The package bears a statement to the effect that the sterility of Oxidized Cellulose cannot be guaranteed if the package bears evidence of damage, or if the package has been previously opened. Oxidized Cellulose meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*.

## Oxidized Regenerated Cellulose

#### DEFINITION

Oxidized Regenerated Cellulose contains NLT 18.0% and NMT 24.0% of carboxyl groups (COOH), calculated on the dried basis. It is sterile.

#### IDENTIFICATION

- **A.**

**Sample solution:** 200 mg in 10 mL of 0.25 N sodium hydroxide

**Analysis 1:** Shake the *Sample solution* for 1 min. Add 10 mL of water, and shake.

**Acceptance criteria 1:** The *Sample solution* shows no more than a slight haze and is substantially free from fibers and foreign particles.

**Analysis 2:** Allow the resulting solution to stand for 10 min.

**Acceptance criteria 2:** Any swollen fibers initially present are no longer visible.

**Analysis 3:** Acidify the resulting solution with 3 N hydrochloric acid.

**Acceptance criteria 3:** A flocculent white precipitate is formed.

#### ASSAY

- **PROCEDURE**

**Sample:** 1 g of Oxidized Regenerated Cellulose, previously dried at 90° for 2 h

#### Titrimetric system

(See *Titrimetry* (541).)

Mode: Direct titration

Titrant: 0.1 N hydrochloric acid VS

Endpoint detection: Visual

**Analysis:** Place the *Sample* in a 250-mL conical flask, add 10 mL of 0.5 N sodium hydroxide VS, swirl to dissolve, and add 100 mL of water. Immediately titrate with *Titrant* to a phenolphthalein endpoint. Perform a blank determination, and note the difference in volumes required. Each mL of the difference in volumes of 0.1 N hydrochloric acid consumed is equivalent to 4.50 mg of carboxyl groups (COOH).

**Acceptance criteria:** 18.0%–24.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.15%

- **LIMIT OF NITROGEN**

*Solution A:* 40 mg/mL of boric acid

*Solution B:* Methyl red TS and bromocresol green TS (1:4)



**Sample:** 1 g, previously dried

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.02 N sulfuric acid VS

**Endpoint detection:** Visual

**Analysis:** Place a 125-mL conical flask, containing 30 mL of *Solution A* and 6 drops of *Solution B*, beneath the condenser of the distillation apparatus so that the tip of the condenser is well below the surface of the resulting solution. To a 500-mL Kjeldahl flask add the *Sample*, and add 1 g of Devarda's alloy, 100 mL of recently boiled water, a small lump of paraffin, and 100 mL of 1 N sodium hydroxide. Connect the Kjeldahl flask to the condenser by a suitable trap bulb. Heat the mixture in the flask until 45–50 mL of distillate has collected in the receiver. Rinse the condenser, and titrate the resulting solution with *Titrant* to a pale pink endpoint that persists for 30 s. Perform a blank determination, and make any necessary correction. Each mL of *Titrant* is equivalent to 0.2801 mg of nitrogen.

**Acceptance criteria:** NMT 0.5%

• **LIMIT OF FORMALDEHYDE**

**Solution A:** Formaldehyde in water (1 in 40,000)

**Standard:** 0.50 mL of *Solution A*

**Sample:** 500 mg

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** Vis

**Analytical wavelength:** 570 nm

**Blank:** Mixture of 0.5 mL of water and 10 mL of chromotropic acid TS

**Analysis:** Transfer the *Sample* to a 500-mL iodine flask. Add 250 mL of water, and allow to stand for NLT 2 h with intermittent shaking. Pipet 0.50 mL each of the supernatant from the resulting solution and the *Standard* into two separate glass-stoppered test tubes. To each test tube add 10 mL of chromotropic acid TS. Stopper the tubes loosely, and heat in a boiling water bath for 30 min. Cool, and determine the absorbance of each solution against the *Blank*.

**Acceptance criteria:** 0.5% CH<sub>2</sub>O; the absorbance of the *Sample* is NMT the *Standard*.

**SPECIFIC TESTS**

• **STERILITY TESTS (71)**

**Sample:** 250 mg

**Analysis:** Proceed as directed in the chapter, adding 0.5 mL of 0.1 N sodium hydroxide to the portions of media used.

**Acceptance criteria:** Meets the requirements

• **LOSS ON DRYING (731)**

**Sample:** 150 mg

**Analysis:** Dry at 90° for 2 h.

**Acceptance criteria:** NMT 15.0%

**ADDITIONAL REQUIREMENTS**

**Change to read:**

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017), protected from direct sunlight. Store at controlled room temperature.
- **LABELING:** The package bears a statement to the effect that the sterility of Oxidized Regenerated Cellulose cannot be guaranteed if the package bears evidence of damage, or if the package has been previously opened. Oxidized Regenerated Cellulose meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*.

## Cellulose Sodium Phosphate

» Cellulose Sodium Phosphate is prepared by phosphorylation of alpha cellulose. It has an inorganic bound phosphate content of not less than 31.0 percent and not more than 36.0 percent, calculated on the dried basis.

**Packaging and storage—**Preserve in well-closed containers.

**pH (791)—**Place 3 g of it in a 100-mL beaker, add 60 mL of water, and stir occasionally for 5 minutes. Filter through a sintered-glass crucible. The pH of the filtrate is between 6.0 and 9.0.

**Loss on drying (731)—**Dry it at 105° for 3 hours: it loses not more than 10.0% of its weight.

**Nitrogen Determination (461), Method I:** not more than 1.0%.

**Calcium binding capacity—**

**Standard calcium solution—**Transfer about 0.33 g of dried calcium carbonate, primary standard grade, accurately weighed, to a 250-mL beaker with the aid of a few mL of water, and dilute with water to about 50 mL. Carefully and dropwise add 2 N hydrochloric acid until all of the solid dissolves, and add 2 drops in excess. Heat the solution to boiling, and boil for 5 minutes. Cool the solution, and transfer to a 1000-mL volumetric flask. Dilute with water to volume, and mix. Calculate the molarity, *M*, of the solution taken by the formula:

$$g / 100.09$$

in which *g* is the weight, in g, of calcium carbonate taken.

**Standard edetate disodium titrant—**Dissolve 10 g of edetate disodium in 100 mL of water. Slowly add alcohol until the first permanent precipitate is formed. Filter, and discard the solid. Add an equal volume of alcohol to the filtrate. Filter the resulting precipitate, discard the filtrate, and wash the residue on the filter, first with acetone, then with ethyl ether. Dry at 80° for 4 days at about 50% relative humidity. Transfer about 3.72 g of this purified edetate disodium, accurately weighed, to a 1000-mL volumetric flask, and dissolve with water. Dilute with water to volume, and mix. Calculate the molarity, *M<sub>s</sub>*, of the solution taken by the formula:

$$w / 372.24$$

in which *w* is the weight, in g, of the purified edetate disodium taken.

**Procedure—**Transfer 0.15 ± 0.02 g of Cellulose Sodium Phosphate, accurately weighed, to a 250-mL beaker. Add 150.0 mL of *Standard calcium solution*, and stir the mixture for 5 minutes on a magnetic stirrer. Filter, discarding the first few mL of the filtrate. To 50.0 mL of the filtrate add about 50 mL of water, 15 mL of 1 N sodium hydroxide, and 300 mg of hydroxy naphthol blue. Titrate with *Standard edetate disodium titrant* to a permanent deep blue color, and designate the number of mL consumed as *V<sub>s</sub>*. Calculate the calcium binding capacity of the undried Cellulose Sodium Phosphate, in mmol per g, by the formula:

$$(150M - 3V_s M_s) / W$$

in which *W* is the weight, in g, of Cellulose Sodium Phosphate taken; and the other terms are as defined above. The calcium binding capacity, calculated on the dried basis, is not less than 1.8 mmol per g.



**Delete the following:**

• **Heavy metals**, Method III (231): 0.004%. • (Official 1-Jan-2018)

**Free phosphate—**

**Standard preparation**—Prepare as directed under *Inorganic bound phosphate*.

**Test preparation**—Transfer about 2 g of Cellulose Sodium Phosphate, accurately weighed, to a 250-mL beaker. Add 100 mL of water, accurately measured, stir, allow to stand for 5 minutes, stir again, and filter through moderately retentive filter paper, collecting the filtrate in a dry flask.

**Procedure**—Transfer 2.0 mL of the *Standard preparation* and 5.0 mL of the *Test preparation* to separate 100-mL volumetric flasks. Proceed as directed in the *Procedure under Inorganic bound phosphate*, beginning with "Treat each of these." Calculate the percentage of free phosphate taken by the formula:

$$(4000 / W)(A_U / A_S)$$

in which *W* is the weight, in mg, of undried Cellulose Sodium Phosphate taken; not more than 3.5%, calculated on the dried basis, is found.

**Sodium content—**

**Standard stock solution**—Dissolve 508.5 mg of sodium chloride, previously dried at 105° for 2 hours, in 100 mL of water, transfer to a 1000-mL volumetric flask, dilute with water to volume, and mix. Each mL of this solution contains 200 µg of sodium.

**Standard preparations**—Transfer 5.0, 10.0, 15.0, and 20.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks, dilute each with water to volume, and mix.

**Test preparation**—Dissolve about 250 mg of Cellulose Sodium Phosphate, accurately weighed, in 10 mL of a mixture of 20 mL of perchloric acid and 15 mL of nitric acid. Heat cautiously to the production of dense, white fumes, cool, transfer to a 1000-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the emission of the *Test preparation* and of each *Standard preparation* at the sodium emission line of 589 nm, with a suitable flame photometer. Plot the emissions of the *Standard preparations* versus their concentration of sodium, and draw a straight line best fitting the four plotted points. From the graph so obtained determine the concentration of sodium in the *Test preparation*. Calculate the percentage of sodium in the undried Cellulose Sodium Phosphate. The content of sodium, calculated on the dried basis, is not less than 9.5% and not more than 13.0%.

**Inorganic bound phosphate—**

**Standard preparation**—Transfer 358.2 mg of monobasic potassium phosphate, primary standard grade, to a 250-mL volumetric flask, dissolve in and dilute with water to volume, and mix. Each mL of this solution contains 1.0 mg of phosphate.

**Test preparation**—Transfer about 250 mg of Cellulose Sodium Phosphate, accurately weighed, to a 250-mL conical flask. Rinse to the bottom with a few mL of water. Add 10 mL of a mixture of 20 mL of perchloric acid and 15 mL of nitric acid. Heat cautiously to the production of dense, white fumes, cool the clear, almost colorless, solution, and transfer to a 100-mL volumetric flask with the aid of water. Dilute with water to volume, and mix.

**Procedure**—Transfer 2.0-mL portions of the *Standard preparation* and the *Test preparation* to separate 100-mL volumetric flasks. Treat each of these and a third flask, providing the blank, as follows: Add 10 mL of 5 N nitric acid, 10.0 mL of ammonium vanadate TS, and about 60 mL of water. Swirl, and add 10.0 mL of a freshly prepared solution of 2.5 g of ammonium molybdate in 50 mL of warm water. Dilute with water to volume, and mix. Concomitantly determine the absorbances,  $A_U$  and  $A_S$ , of the solutions from the

*Standard preparation* and the *Test preparation*, respectively, at 400 nm with a suitable spectrophotometer, using the reagent blank to set the instrument. Calculate the percentage of total phosphate taken by the formula:

$$(10,000 / W)(A_U / A_S)$$

in which *W* is the weight, in mg, of Cellulose Sodium Phosphate taken. Calculate the percentage of inorganic bound phosphate in the undried Cellulose Sodium Phosphate by subtracting from this result the percentage of *Free phosphate*.

## Cellulose Sodium Phosphate for Oral Suspension

» Cellulose Sodium Phosphate for Oral Suspension contains Cellulose Sodium Phosphate. It has an inorganic bound phosphate content of not less than 28.0 percent and not more than 36.0 percent calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers. Store in a refrigerator.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 10.0% of its weight.

**Calcium binding capacity—**

**Standard calcium solution** and **Standard edetate disodium titrant**—Proceed as directed for *Calcium binding capacity under Cellulose Sodium Phosphate*.

**Procedure**—Transfer an accurately weighed amount of Oral Suspension, equivalent to about 0.15 g of cellulose sodium phosphate, to a 250-mL beaker. Proceed as directed for *Procedure in Calcium binding capacity under Cellulose Sodium Phosphate*, beginning with "Add 150.0 mL of *Standard calcium solution*." Calculate the calcium binding capacity, in mmol per g, of the portion of undried Cellulose Sodium Phosphate for Oral Suspension taken by the formula:

$$(150M_S - 3V_S M_S) / W$$

in which *W* is the weight, in g, of Cellulose Sodium Phosphate for Oral Suspension taken; and the other terms are as defined therein: not less than 1.8 mmol per g, calculated on the dried basis, is found.

**Free phosphate—**

**Standard preparation**—Proceed as directed for *Free phosphate under Cellulose Sodium Phosphate*.

**Test preparation**—Transfer an accurately weighed amount of Cellulose Sodium Phosphate for Oral Suspension, equivalent to about 2 g of cellulose sodium phosphate, to a 250-mL beaker. Proceed as directed for *Free phosphate under Cellulose Sodium Phosphate*, beginning with "Add 100 mL of water."

**Procedure**—Proceed as directed for *Free phosphate under Cellulose Sodium Phosphate*. Calculate the percentage of free phosphate in the portion of Cellulose Sodium Phosphate for Oral Suspension taken by the formula:

$$(4000 / W)(A_U / A_S)$$

in which *W* is the weight, in mg, of undried Cellulose Sodium Phosphate for Oral Suspension taken; and the other terms are as defined therein: not more than 6.0%, calculated on the dried basis, is found.

**Inorganic bound phosphate—**

**Standard preparation**—Proceed as directed for *Inorganic bound phosphate under Cellulose Sodium Phosphate*.



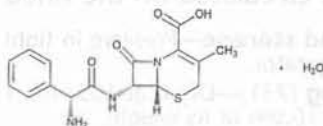
**Test preparation**—Transfer an accurately weighed amount of Cellulose Sodium Phosphate for Oral Suspension, equivalent to about 250 mg of cellulose sodium phosphate, to a 250-mL conical flask. Proceed as directed for *Test preparation in Inorganic bound phosphate under Cellulose Sodium Phosphate*, beginning with "Rinse to the bottom."

**Procedure**—Proceed as directed for *Inorganic bound phosphate under Cellulose Sodium Phosphate*. Calculate the percentage of total phosphate in the portion of Cellulose Sodium Phosphate for Oral Suspension taken by the formula:

$$(10,000 / W)(A_U / A_S)$$

in which *W* is the weight, in mg, of Cellulose Sodium Phosphate for Oral Suspension taken; and the other terms are as defined therein. Calculate the percentage of inorganic bound phosphate in the portion of undried Cellulose Sodium Phosphate for Oral Suspension by subtracting from this result the percentage of free phosphate.

## Cephalexin



$C_{16}H_{17}N_3O_4S \cdot H_2O$  365.40

$C_{16}H_{17}N_3O_4S$  347.40

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-methyl-8-oxo-, monohydrate, [6R-[6 $\alpha$ ,7 $\beta$  (R\*)]]-; (6R,7R)-7-[(R)-2-Amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate [23325-78-2].

Anhydrous [15686-71-2].

### DEFINITION

Cephalexin has a potency of NLT 950  $\mu$ g/mg and NMT 1030  $\mu$ g/mg of  $C_{16}H_{17}N_3O_4S$ , calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Mobile phase:** 0.985 g/L of sodium-1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of 3.0  $\pm$  0.1

**Standard stock solution:** 1 mg/mL of USP Cephalexin RS in water

**Standard solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*

**Sample stock solution:** 1 mg/mL of Cephalexin in water

**Sample solution:** 0.4 mg/mL of Cephalexin in *Mobile phase* from *Sample stock solution*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1 of low acidity

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in  $\mu$ g, of cephalexin

( $C_{16}H_{17}N_3O_4S$ ) per mg of the Cephalexin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cephalexin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cephalexin in the *Sample solution* (mg/mL)

$P$  = potency of cephalexin in USP Cephalexin RS ( $\mu$ g/mg)

**Acceptance criteria:** 950–1030  $\mu$ g/mg on the anhydrous basis

### IMPURITIES

#### Organic Impurities

##### • PROCEDURE 1

**Solution A:** Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 1000 mL of water and 15 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5  $\pm$  0.1.

**Solution B:** Dissolve 1 g of sodium 1-pentanesulfonate in a mixture of 300 mL of water and 15 mL of triethylamine. Adjust with phosphoric acid to a pH of 2.5  $\pm$  0.1, and add 350 mL of acetonitrile and 350 mL of methanol.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1	100	0
33.3	0	100
34.3	0	100

**Diluent:** 18 mg/mL of monobasic potassium phosphate in water

**Standard solutions:** 0.08 mg/mL and 0.16 mg/mL of cephalexin ( $C_{16}H_{17}N_3O_4S$ ) from USP Cephalexin RS in *Diluent*, taking into account the stated potency of the USP Cephalexin RS

**Sample solution:** 5 mg/mL of Cephalexin in *Diluent*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1 of low acidity

**Flow rate:** 1 mL/min

**Injection size:** 20  $\mu$ L

### Analysis

**Samples:** *Standard solutions* and *Sample solution*

Plot the responses of the cephalexin peaks from the *Standard solutions* versus their concentrations, calculated on the anhydrous basis, in mg/mL, and draw a straight line through the two points and zero. From the line and the peak responses of the *Sample solution*, determine the concentration, *I*, in mg/mL, of each cephalexin-related substance of the *Sample solution* other than the cephalexin peak.



Calculate the percentage of each cephalexin-related substance:

$$\text{Result} = I/C \times 100$$

- $I$  = concentration of each cephalexin-related substance in the *Sample solution* as determined from the calibration curve (mg/mL)  
 $C$  = concentration of cephalexin from the *Sample solution* (mg/mL)

#### Acceptance criteria

Individual impurities: NMT 1.0% of any individual cephalexin-related substance  
 Total impurities: NMT 5.0%

- **PROCEDURE 2: DIMETHYLANILINE (223):** Meets the requirement

#### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation (781S):** +149° to +158°  
*Sample solution:* 5 mg/mL, in pH 4.4 neutralized phthalate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)
- **CRYSTALLINITY (69S):** Meets the requirements
- **PH (791):** 3.0–5.5, in an aqueous suspension containing 50 mg/mL
- **WATER DETERMINATION, Method I (921):** 4.0%–8.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
 USP Cephalexin RS

## Cephalexin Capsules

#### DEFINITION

Cephalexin Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cephalexin ( $C_{16}H_{17}N_3O_4S$ ).

#### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

*Mobile phase:* 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of  $3.0 \pm 0.1$

*Standard stock solution:* 1 mg/mL of USP Cephalexin RS in water

*Standard solution:* 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*

*Sample stock solution:* Equivalent to 1 mg/mL of cephalexin from combined contents of NLT 20 Capsules in water. Sonicate, if necessary, to dissolve the cephalexin. Filter, if necessary, to obtain a clear solution.

*Sample solution:* 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*

*Chromatographic system*

(See *Chromatography (621)*, *System Suitability*.)

*Mode:* LC

*Detector:* UV 254 nm

*Column:* 4.6-mm  $\times$  25-cm; packing L1 of low acidity

*Flow rate:* 1.5 mL/min

*Injection size:* 20  $\mu$ L

*System suitability*

*Sample:* *Standard solution*

*Suitability requirements*

Relative standard deviation: NMT 2.0%

*Analysis*

*Samples:* *Standard solution* and *Sample solution*

Calculate the percentage of cephalexin ( $C_{16}H_{17}N_3O_4S$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cephalexin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cephalexin in the *Sample solution* (mg/mL)

$P$  = potency of cephalexin in USP Cephalexin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

Acceptance criteria: 90.0%–120.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

*Medium:* Water; 900 mL

*Apparatus 1:* 100 rpm

*Time:* 30 min

*Standard solution:* 20  $\mu$ g/mL of USP Cephalexin RS in *Medium*

*Sample solution:* Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration of about 20  $\mu$ g/mL.

*Spectrometric conditions*

(See *Ultraviolet-Visible Spectroscopy (857)*.)

*Mode:* UV

*Analytical wavelength:* 262 nm

*Analysis*

*Samples:* *Standard solution* and *Sample solution*

*Tolerances:* NLT 80% (Q) of the labeled amount of cephalexin ( $C_{16}H_{17}N_3O_4S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
 USP Cephalexin RS

## Cephalexin for Oral Suspension

#### DEFINITION

Cephalexin for Oral Suspension is a dry mixture of Cephalexin and one or more suitable buffers, colors, diluents, and flavors. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of  $C_{16}H_{17}N_3O_4S$  per mL when constituted as directed in the labeling.

#### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

*Mobile phase:* 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine,



and water (20:10:3:170), adjusted with phosphoric acid to a pH of  $3.0 \pm 0.1$ .

**Standard stock solution:** 1 mg/mL of USP Cephalixin RS in water

**Standard solution:** Mix 10.0 mL of *Standard stock solution* with 15.0 mL of *Mobile phase*.

**Sample stock solution:** Nominally equivalent to 1 mg/mL of cephalixin from Oral Suspension, constituted as directed in the labeling, freshly mixed and free from air bubbles. Sonicate, if necessary, to assure complete dissolution of the cephalixin. Filter, if necessary, to obtain a clear solution.

**Sample solution:** Mix 10.0 mL of *Sample stock solution* and 15.0 mL of *Mobile phase*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1 of low acidity

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cephalixin ( $C_{16}H_{17}N_3O_4S$ ) in each mL of the constituted Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cephalixin RS in the *Standard stock solution* (mg/mL)

$C_U$  = nominal concentration of cephalixin from the *Sample stock solution* (mg/mL)

$P$  = potency of USP Cephalixin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–120.0%

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): For solid packaged in single-unit containers: meets the requirements
- **DELIVERABLE VOLUME** (698): Meets the requirements

#### SPECIFIC TESTS

- **pH** (791): 3.0–6.0, constituted as directed in the labeling

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Cephalixin RS

### Cephalixin Tablets

#### DEFINITION

Cephalixin Tablets are prepared from Cephalixin or Cephalixin Hydrochloride. They contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cephalixin ( $C_{16}H_{17}N_3O_4S$ ).

#### IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Mobile phase:** 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine,

and water (20:10:3:170). Adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ .

**Standard stock solution:** 1 mg/mL of USP Cephalixin RS in water

**Standard solution:** 0.4 mg/mL of cephalixin in *Mobile phase* from *Standard stock solution*

**Sample stock solution:** Equivalent to 1 mg/mL of cephalixin from combined contents of powdered Tablets (NLT 20) in water. Sonicate, if necessary, to assure complete dissolution of the cephalixin. Filter, if necessary, to obtain a clear solution.

**Sample solution:** 0.4 mg/mL of cephalixin in *Mobile phase* from *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1 of low acidity

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cephalixin ( $C_{16}H_{17}N_3O_4S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cephalixin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cephalixin in the *Sample solution* (mg/mL)

$P$  = potency of cephalixin in USP Cephalixin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–120.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**For Cephalixin**

**Medium:** Water; 900 mL

**Apparatus 1:** Use 40-mesh cloth and 100 rpm

**Time:** 30 min

**Standard solution:** 20  $\mu$ g/mL of USP Cephalixin RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute, if necessary, with *Medium* to a concentration that is similar to the *Standard solution*.

#### Spectrometric conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV

**Analytical wavelength:** 262 nm

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** NLT 80% (Q) of the labeled amount of cephalixin ( $C_{16}H_{17}N_3O_4S$ ) is dissolved.

**For Cephalixin hydrochloride**

**Medium, Standard solution, Sample solution, Spectrometric conditions, and Analysis:** Proceed as directed *For Cephalixin*.

**Apparatus 1:** Use 10-mesh cloth and 150 rpm.

**Time:** 45 min

**Tolerances:** NLT 75% (Q) of the labeled amount of cephalixin ( $C_{16}H_{17}N_3O_4S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** The label states whether the Tablets contain Cephalexin or Cephalexin Hydrochloride.
- **USP REFERENCE STANDARDS (11)**  
USP Cephalexin RS

**Cephalexin Tablets for Oral Suspension****DEFINITION**

Cephalexin Tablets for Oral Suspension contain NLT 90.0% and NMT 110.0% of the labeled amount of cephalexin ( $C_{16}H_{17}N_3O_4S$ ).

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE**

**Mobile phase:** 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of  $3.0 \pm 0.1$ .

**Standard stock solution:** 1 mg/mL of USP Cephalexin RS in water

**Standard solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*

**Sample stock solution:** Nominally equivalent to 1 mg/mL of cephalexin from combined contents of NLT 20 powdered Tablets for Oral Suspension in water. Pass a portion of the solution through a filter having a 1- $\mu$ m or finer pore size.

**Sample solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1 of low acidity

**Flow rate:** 1.5 mL/min

**Injection size:** 20  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cephalexin ( $C_{16}H_{17}N_3O_4S$ ) in each Tablet for Oral Suspension:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cephalexin RS in the *Standard stock solution* (mg/mL)

$C_U$  = nominal concentration of cephalexin in the *Sample stock solution* (mg/mL)

$P$  = potency of cephalexin in USP Cephalexin RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **DISINTEGRATION (701):** Tablets for Oral Suspension disintegrate in 3 min, using water at  $20 \pm 5^\circ$ .

**DISSOLUTION (711)**

**Medium:** Water; 900 mL

**Apparatus 1:** Use 40-mesh cloth and 100 rpm.

**Time:** 30 min

**Standard solution:** 20  $\mu$ g/mL of USP Cephalexin RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary, to a concentration of about 20  $\mu$ g/mL.

**Spectrometric conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV

**Analytical wavelength:** 262 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** NLT 80% (Q) of the labeled amount of cephalexin ( $C_{16}H_{17}N_3O_4S$ ) is dissolved.

- **DISPERSION FINENESS:** Place 2 Tablets for Oral Suspension in 100 mL of water, and stir until completely dispersed. A smooth dispersion is obtained that passes through a No. 25 sieve.
- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Cephalexin RS

**Cephalexin Hydrochloride**

$C_{16}H_{17}N_3O_4S \cdot HCl \cdot H_2O$  401.87  
5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(aminophenylacetyl)amino]-3-methyl-8-oxo-, monohydrochloride, monohydrate, [6R-[6 $\alpha$ ,7 $\beta$  (R\*)]]-; (6R,7R)-7-[(2R)-2-Amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, monohydrochloride, monohydrate; 7-(D-2-Amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid hydrochloride monohydrate [105879-42-3].

**DEFINITION**

Cephalexin Hydrochloride contains the equivalent of NLT 800  $\mu$ g/mg and NMT 880  $\mu$ g/mg of cephalexin ( $C_{16}H_{17}N_3O_4S$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Chloride (191):** 10 mg/mL meets the requirements

**ASSAY****PROCEDURE**

**Mobile phase:** 0.985 g/L of sodium 1-pentanesulfonate in a mixture of acetonitrile, methanol, triethylamine, and water (20:10:3:170), adjusted with phosphoric acid to a pH of  $3.0 \pm 0.1$ .

**Standard stock solution:** 1 mg/mL of USP Cephalexin RS in water

**Standard solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Standard stock solution*

**Sample stock solution:** 1.15 mg/mL of Cephalexin Hydrochloride in water

**Sample solution:** 0.4 mg/mL of cephalexin in *Mobile phase* from *Sample stock solution*



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1 of low acidity

Flow rate: 1.5 mL/min

Injection size: 20 µL

**System suitability**Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of cephalexin

(C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S) in each mg of Cephalexin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Cephalexin RS in the *Standard stock solution* (mg/mL) $C_U$  = concentration of Cephalexin Hydrochloride from the *Sample stock solution* (mg/mL) $P$  = potency of cephalexin in USP Cephalexin RS (µg/mg)

Acceptance criteria: 800–880 µg/mg

**IMPURITIES****Organic Impurities**• **PROCEDURE 1****Solution A:** 1 g of sodium 1-pentanesulfonate in a mixture of 1000 mL of water and 15 mL of triethylamine.

Adjust with phosphoric acid to a pH of 2.5 ± 0.1.

**Solution B:** 1 g of sodium 1-pentanesulfonate in a mixture of 300 mL of water and 15 mL of triethylamine.

Adjust with phosphoric acid to a pH of 2.5 ± 0.1, and add 350 mL of acetonitrile and 350 mL of methanol.

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
1	100	0
33.3	0	100
34.3	0	100

**Diluent:** 18 mg/mL of monobasic potassium phosphate in water**Standard solutions:** 0.08 mg/mL and 0.16 mg/mL of cephalexin (C<sub>16</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub>S) from USP Cephalexin RS in *Diluent*, taking into account the stated potency of the USP Cephalexin RS**Sample solution:** 6 mg/mL of Cephalexin Hydrochloride in *Diluent***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1 of low acidity

Flow rate: 1 mL/min

Injection size: 20 µL

**Analysis**Samples: *Standard solutions* and *Sample solution*

Plot the responses of the cephalexin peaks of the *Standard solutions* versus their concentrations, calculated on the anhydrous basis, in mg/mL, and draw a straight line through the two points and zero. From the line and the peak responses of the *Sample solution*, determine the concentration,  $I$ , in mg/mL, of each cephalexin-related substance from the *Sample solution* other than the cephalexin peak.

Calculate the percentage of each cephalexin-related substance represented by each peak of the *Sample solution*, other than the cephalexin peak.

$$\text{Result} = (I/C) \times 100$$

 $I$  = concentration of each cephalexin-related substance other than cephalexin in the *Sample solution* (mg/mL) $C$  = concentration of cephalexin from the *Sample solution* (mg/mL)**Acceptance criteria****Individual impurities:** NMT 1.0% of any individual cephalexin-related substance is found.**Total impurities:** NMT 5.0%

- **PROCEDURE 2: DIMETHYLANILINE** (223): Meets the requirement

**SPECIFIC TESTS**

- **CRYSTALLINITY** (695): Meets the requirements

- **pH** (791): 1.5–3.0, in a solution containing 10 mg/mL

- **WATER DETERMINATION, Method I** (921): 3.0%–6.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.

- **USP REFERENCE STANDARDS** (11)

USP Cephalexin RS

**Cephalothin Injection**

» Cephalothin Injection contains an amount of Cephalothin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephalothin (C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>).

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*. Maintain in the frozen state.

**Labeling**—It meets the requirements for *Labeling* (7), *Labels and Labeling for Injectable Products*. The label states that it is to be thawed just prior to use, describes conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.

**USP Reference standards** (11)—

USP Cephalothin Sodium RS

USP Endotoxin RS

**Bacterial Endotoxins Test** (85)—It contains not more than 0.13 USP Endotoxin Unit per mg of cephalothin.

**pH** (791): between 6.0 and 8.5.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It responds to the *Identification test A* under *Cephalothin Sodium* and meets the requirements for *Sterility* under *Cephalothin for Injection*.

**Assay**—

**Mobile phase, Standard preparation, Resolution solution, and Chromatographic system**—Proceed as directed in the *Assay* under *Cephalothin Sodium*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of cephalothin (C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>), to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Cephalothin Sodium*. Calculate the quantity, in µg, of



cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ) in each mL of the Injection taken by the formula:

$$100(CP / V)(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Cephalothin Sodium RS, in the *Standard preparation*;  $P$  is the assigned potency, in  $\mu$ g of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ) per mg, of USP Cephalothin Sodium RS;  $V$  is the volume, in mL, of Injection taken to prepare the *Assay preparation*; and  $r_u$  and  $r_s$  are the cephalothin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephalothin for Injection

» Cephalothin for Injection contains an amount of Cephalothin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ). It may contain Sodium Bicarbonate.

### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

**USP Reference standards** (11)—  
USP Cephalothin Sodium RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

**Specific rotation** (781S): between  $+124^\circ$  and  $+134^\circ$ , calculated on the dried and sodium bicarbonate-free basis.

**Test solution**: a known amount of specimen, equivalent to about 50 mg of cephalothin, per mL, in water.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.13 USP Endotoxin Unit per mg of cephalothin.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**Uniformity of dosage units** (905): meets the requirements.

**Procedure for content uniformity**—Perform the Assay on individual containers using *Assay preparation 1* or *Assay preparation 2*, or both, as appropriate.

**pH** (791): between 6.0 and 8.5, in the solution constituted as directed in the labeling.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Content of sodium bicarbonate (if present)**—Dissolve about 1 g of it, accurately weighed, in 50 mL of water. Add methyl orange TS, and titrate with 0.1 N sulfuric acid VS. Each mL of 0.1 N sulfuric acid is equivalent to 8.401 mg of  $NaHCO_3$ . Calculate the percentage of sodium bicarbonate, and use the value obtained to calculate the *Specific rotation* on the dried and sodium bicarbonate-free basis.

**Other requirements**—It meets the requirements for *Identification test A* and *Loss on drying* under *Cephalothin Sodium*. It meets also the requirements *Labeling* (7), *Labels and Labeling for Injectable Products*.

## Assay—

*Mobile phase, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under *Cephalothin Sodium*.

**Standard preparation**—Dissolve a suitable quantity of USP Cephalothin Sodium RS, accurately weighed, in *Mobile phase* to obtain a solution having a known concentration of about 1 mg of cephalothin per mL.

**Assay preparation 1** (where it is represented as being in a single-dose container)—Constitute Cephalothin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Mobile phase* to obtain a solution having a concentration of about 1 mg of cephalothin per mL.

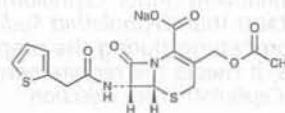
**Assay preparation 2** (where the label states the quantity of cephalothin in a given volume of constituted solution)—Constitute 1 container of Cephalothin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured portion of the constituted solution quantitatively with *Mobile phase* to obtain a solution having a concentration of about 1 mg of cephalothin per mL.

**Procedure**—Proceed as directed in the Assay under *Cephalothin Sodium*. Calculate the quantity, in mg, of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ) in the container, and in the portion of constituted solution taken by the formula:

$$(L / D)(CP / 1000)(r_u / r_s)$$

in which  $L$  is the labeled quantity of cephalothin in the container, or in the volume of constituted solution taken;  $D$  is the concentration, in mg per mL, of cephalothin in *Assay preparation 1* or in *Assay preparation 2*, on the basis of the labeled quantity in the container, or in the portion of constituted solution taken, respectively, and the extent of dilution; and the other terms are as defined therein. Where the test for *Uniformity of dosage units* has been performed using the *Procedure for content uniformity*, use the average of these determinations as the Assay value.

## Cephalothin Sodium



$C_{16}H_{15}N_2NaO_6S_2$  418.42

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyloxy)methyl]-8-oxo-7-[(2-thienylacetyl)amino]-, monosodium salt, (6R-trans)-.

Monosodium (6R,7R)-3-(hydroxymethyl)-8-oxo-7-[2-(2-thienyl)-acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate acetate (ester) [58-71-9].

» Cephalothin Sodium contains the equivalent of not less than 850  $\mu$ g of cephalothin ( $C_{16}H_{16}N_2O_6S_2$ ) per mg, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.



**USP Reference standards** (11)—

USP Cephalothin Sodium RS

USP Endotoxin RS

**Identification**—**A:** Ultraviolet Absorption (197U)—

Solution: 25 µg per mL.

Medium: water.

**B:** It responds to the tests for Sodium (191).**Specific rotation** (781S): between +124° and +134°.**Test solution:** a known amount of specimen, equivalent to about 50 mg of cephalothin, per mL, in water.**Crystallinity** (69S): meets the requirements.**pH** (791): between 4.5 and 7.0, in a solution containing 250 mg per mL.**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 1.5% of its weight.**Chromatographic purity**—**Mobile phase, Resolution solution, and Chromatographic system**—Proceed as directed in the Assay.**Standard solution**—Use the *Standard preparation*, prepared as directed in the Assay, transfer 1.0 mL to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.**Test solution**—Use the *Assay preparation* prepared as directed in the Assay.**Procedure**—Proceed as directed for the *Procedure* in the Assay, except to inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* and to continue the chromatography of the *Test solution* for at least 4 times the retention time of the main cephalothin peak. The area of any peak in the chromatogram obtained from the *Test solution*, except the main peak, is not greater than the area of the main peak in the chromatogram obtained from the *Standard solution* (1.0%), and the sum of the areas of any such peaks is not greater than 3 times the area of the main peak in the chromatogram obtained from the *Standard solution* (3.0%). [NOTE—Any peak in the chromatogram obtained from the *Test solution* with an area less than one-tenth that of the main peak in the chromatogram obtained from the *Standard solution* is disregarded.]**Other requirements**—Where the label states that Cephalothin Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cephalothin for Injection*. Where the label states that *Cephalothin Sodium* must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cephalothin for Injection*.**Assay**—**Mobile phase**—Dissolve 17 g of sodium acetate in 790 mL of water, add 0.6 mL of glacial acetic acid, and if necessary adjust with 0.1 N sodium hydroxide or glacial acetic acid to a pH of 5.9 ± 0.1. Add 150 mL of acetonitrile and 70 mL of alcohol, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).**Standard preparation**—Dissolve an accurately weighed quantity of USP Cephalothin Sodium RS quantitatively in *Mobile phase* to obtain a solution having a known concentration of about 1 mg per mL.**Resolution solution**—Heat a 5-mL portion of the *Standard preparation* in a water bath at 90° for 10 minutes. Cool the solution, and immediately inject a portion of it into the chromatograph as directed under *Chromatographic system*.**Assay preparation**—Transfer about 25 mg of Cephalothin Sodium, accurately weighed, to a 25-mL volumetric flask, add about 15 mL of *Mobile phase*, swirl to dissolve, dilute with *Mobile phase* to volume, and mix.**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detectorand a 4.6-mm × 25-cm column that contains 5 µm packing L1 and is maintained at a constant temperature of about 40°. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the resolution between the two principal peaks is not less than 9.0. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the tailing factor is not more than 1.8, and the relative standard deviation for replicate injections is not more than 1.0%.**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in µg, of cephalothin (C<sub>16</sub>H<sub>16</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>) in each mg of Cephalothin Sodium taken by the formula:

$$25(CP / W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cephalothin Sodium RS in the *Standard preparation*; P is the assigned potency, in µg of cephalothin per mg, of USP Cephalothin Sodium RS; W is the quantity, in mg, of Cephalothin Sodium taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the cephalothin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.**Cephapirin for Injection**

» Cephapirin for Injection contains an amount of Cephapirin Sodium equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephapirin.

**Change to read:****Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for constitution* (CN 1-May-2017).**USP Reference standards** (11)—

USP Cephapirin Sodium RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.**Bacterial Endotoxins Test** (85)—It contains not more than 0.17 USP Endotoxin Unit per mg of cephapirin.**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.**Particulate Matter in Injections** (788) : meets the requirements for small-volume injections.**Other requirements**—It responds to the *Identification* tests and meets the requirements for *Crystallinity*, *pH*, and *Water* under *Cephapirin Sodium*. It meets also the requirements for *Uniformity of Dosage Units* (905) and *Labeling* (7), *Labels and Labeling for Injectable Products*.**Assay**—**Mobile phase**—Prepare a filtered and degassed mixture of water, dimethylformamide, glacial acetic acid, and 11.7 N potassium hydroxide (1834:160:4:2). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Increase the proportion of dimethylformamide to decrease the retention time of cephapirin.



**Resolution solution**—Prepare a solution of Cephalixin Sodium in pH 2.0 hydrochloric acid buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) containing about 1 mg per mL. Place 10 mL of this solution in a test tube, and heat at 95° for 10 minutes, accurately timed. Promptly cool the tube in an ice water bath. Dilute 5 mL of the cooled solution with *Mobile phase* to obtain 50 mL of *Resolution solution*.

**Standard preparation**—Transfer about 21 mg of USP Cephalixin Sodium RS, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.2 mg of cephalixin per mL.

**Assay preparation 1** (where it is packaged for dispensing and is represented as being in a single-dose container)—Constitute Cephalixin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing the equivalent of about 0.2 mg of cephalixin per mL.

**Assay preparation 2** (where the label states the quantity of cephalixin in a given volume of constituted solution)—Constitute Cephalixin for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing the equivalent of about 0.2 mg of cephalixin per mL. [NOTE—Use the *Standard preparation* and the *Assay preparation* within 1 hour.]

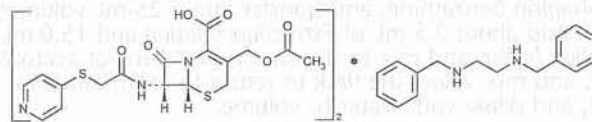
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution, *R*, between the cephalixin peak and the peak having a retention time of about 0.9 relative to that of cephalixin is not less than 0.9. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.9 for cephalixin lactone and 1.0 for cephalixin; the column efficiency determined from the cephalixin peak is not less than 1200 theoretical plates; the tailing factor for the cephalixin peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the appropriate *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephalixin (C<sub>17</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub>) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(L/D)(CP/1000)(r_u/r_s)$$

in which *L* is the labeled quantity, in mg, of cephalixin in the single-dose container, or in the volume of constituted solution taken; *D* is the concentration, in mg per mL, of cephalixin in *Assay preparation 1* or in *Assay preparation 2*, on the basis of the labeled quantity in the container, or in the portion of constituted solution taken, respectively, and the extent of dilution; *C* is the concentration, in mg per mL, of USP Cephalixin Sodium RS in the *Standard preparation*; *P* is the potency, in µg of cephalixin per mg, of USP Cephalixin Sodium RS; and *r<sub>u</sub>* and *r<sub>s</sub>* are the cephalixin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephalixin Benzathine



(C<sub>17</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub>)<sub>2</sub> · C<sub>16</sub>H<sub>20</sub>N<sub>2</sub> 1087.27

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyl-oxy)methyl]-8-oxo-7-[[4-(pyridinylthio)acetyl]amino]-, (6*R*-*trans*)-, compd. with *N,N'*-bis(phenylmethyl)-1,2-ethanediamine (2:1). (6*R*,7*R*)-3-(hydroxymethyl)-8-oxo-7-[2-(4-pyridylthio)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid compound with *N,N'*-dibenzylethylenediamine (2:1) [97468-37-6].

» Cephalixin Benzathine contains the equivalent of not less than 715 µg and not more than 820 µg of cephalixin (C<sub>17</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub>) per mg.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Cephalixin Benzathine RS

USP Cephalixin Sodium RS

**Identification, Infrared Absorption** (197K).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 4.0 and 7.0, in a suspension (1 in 10).

**Water Determination, Method I** (921): not more than 5.0%.

**Benzathine content**—Using about 1 g of Cephalixin Benzathine, accurately weighed, proceed as directed in the test for *Benzathine content* under *Penicillin G Benzathine*: between 20.0% and 24.0% of benzathine (C<sub>16</sub>H<sub>20</sub>N<sub>2</sub>), calculated on the anhydrous basis, is found.

**Assay**—

**Solution A**—Transfer about 26.2 mL of acetic acid and about 99.12 g of potassium acetate to a 4-L volumetric flask. Add 2000 mL of water, and mix to dissolve. Dilute with water to volume, and pass through a 0.45-µm nylon filter.

**Solution B**—Use acetonitrile.

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments, if necessary (see *System Suitability* under *Chromatography* (621)).

**Extraction solution**: a mixture of 400 mL of acetic acid and 600 mL of water.

**Dilution buffer**—Dissolve about 205 g of potassium acetate in about 800 mL of water. Adjust with acetic acid to a pH of 7.5 to 8.2. Dilute with water to 1000 mL, and pass through a 0.45-µm nylon filter.

**10% Acetic acid solution**—Add about 10.0 mL of acetic acid to a 100-mL volumetric flask. Mix, and dilute with water to volume.

**System suitability solution**—Dissolve an accurately weighed quantity of USP Cephalixin Sodium RS in 10% *Acetic acid solution* to prepare a solution containing a known concentration of about 2.0 mg per mL. Heat the solution at 50° for 12 to 18 hours.

**Standard preparation**—In duplicate, accurately weigh about 50 mg of USP Cephalixin Sodium RS, and transfer into a 25-mL volumetric flask. Add about 2.5 mL of *Extraction solution* and about 15.0 mL of *Dilution buffer*, and agitate to dissolve. Add 7.0 mL of acetonitrile, and mix well.



Allow the solution to return to room temperature, and dilute with water to volume.

**Assay preparation**—In duplicate, weigh about 60 mg of Cephapirin Benzathine, and transfer into a 25-mL volumetric flask. Add about 2.5 mL of *Extraction solution* and 15.0 mL of *Dilution buffer*, and mix to dissolve. Add 7.0 mL of acetonitrile, and mix. Allow the flask to return to room temperature, and dilute with water to volume.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 260-nm detector, a 3.2-mm × 15-mm guard column that contains 7-μm packing L1 and a 3.9-mm × 15-cm analytical column that contains 4-μm packing L1. The flow rate is about 2.0 mL per minute, and the columns are heated to 40°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–6	91.5	8.5	isocratic
6–10	91.5→80.0	8.5→20.0	linear
10–12	80.0	20.0	isocratic
12	80.0→91.5	20.0→8.5	return to initial
12–21	91.5	8.5	re-equilibration

Chromatograph the *System suitability solution* and the *Standard preparation*, and record the peak heights and valleys as directed for *Procedure*. Using the results from the *System suitability solution*, calculate the percentage of the height of the valley taken by the formula:

$$100(r_v / r_i)$$

in which  $r_v$  is the height of the valley between cephapirin and any impurity; and  $r_i$  is the impurity peak height. The percentage of the height of the valley is not more than 25% for the impurity peaks adjacent to the cephapirin peak. [NOTE—The *System suitability solution* is acceptable as long as the cephapirin peak is larger than the two peaks on either side of the cephapirin peak.] The relative standard deviation for replicate injections of the *Standard preparation* is not more than 3.0%.

**Procedure**—Separately inject equal volumes (about 2 μL) of the duplicate *Standard preparation* and the duplicate *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in μg, of  $C_{17}H_{17}N_3O_6S_2$  in each mg of Cephapirin Benzathine taken by the formula:

$$P(W_s / W_u)(V_u / V_s)(r_u / r_s)$$

in which  $P$  is the assigned potency, in μg of cephapirin per mg, of USP Cephapirin Sodium RS;  $W_s$  and  $W_u$  are the quantities of USP Cephapirin Sodium RS and Cephapirin Benzathine, in mg, used to prepare the *Standard preparation* and the *Assay preparation*, respectively;  $V_s$  and  $V_u$  are the final volumes, in mL, of the *Standard preparation* and the *Assay preparation*, respectively; and  $r_u$  and  $r_s$  are the average peak areas of the cephapirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephapirin Benzathine Intramammary Infusion

» Cephapirin Benzathine Intramammary Infusion is a suspension of Cephapirin Benzathine in a

suitable vegetable oil vehicle. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled quantity of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ). It contains a suitable dispersing agent.

**Packaging and storage**—Preserve in well-closed unit-dose disposable syringes at controlled room temperature.

**Labeling**—Label Intramammary Infusion to indicate that it is for veterinary use only.

### USP Reference standards (11)—

USP Cephapirin Benzathine RS

USP Cephapirin Sodium RS

**Identification, Infrared Absorption** (197K)—Prepare the test specimen as follows. Transfer the contents of 1 syringe of Intramammary Infusion to a 50-mL centrifuge tube, add 25 mL of toluene, mix for about 1 minute, and centrifuge. Remove and discard the toluene layer without disturbing the residue in the centrifuge tube. Wash the residue with two 25-mL portions of toluene. Dry the residue in vacuum at 60°, and use the dried residue as the test specimen. Mix the dried residue with 9 parts of potassium bromide, and record the IR spectrum, using the diffuse reflectance technique: the IR absorption spectrum so obtained corresponds to that of a similar dispersion of USP Cephapirin Benzathine RS in potassium bromide.

**Water Determination, Method I** (921): not more than 1.0%, 10 mL of Intramammary Infusion being tested.

### Assay—

*Solution A, Solution B, Mobile phase, Extraction solution, Dilution buffer, 10% Acetic acid solution, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under Cephapirin Benzathine.

**Assay preparation**—Express the entire contents of a syringe of the Intramammary Infusion into a centrifuge tube. For each mL of Intramammary Infusion, add 1.0 *n*-heptane and 1.5 mL of *Extraction solution*, cap, and vortex at high speed for 5 minutes. Centrifuge for 5 minutes at a sufficient speed to break the emulsion. Remove the aqueous layer, and pass through a 0.45-μm nylon filter, discarding the first 0.5 mL. Transfer 2.5 mL of the filtered aqueous phase into a 25-mL volumetric flask that contains a solution composed of 15.0 mL of *Dilution buffer* and 7.0 mL of acetonitrile. Add water to volume, and mix well to obtain a single phase.

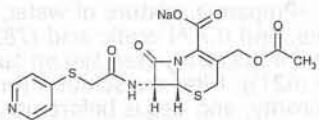
**Procedure**—Separately inject equal volumes (about 2 μL) of the duplicate *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the quantity, in mg, of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ) in each syringe of Intramammary Infusion taken by the formula:

$$15PW(V_u / V_s)(r_u / r_s)$$

in which  $P$  is the assigned potency, in μg cephapirin per mg, of USP Cephapirin Sodium RS;  $W$  is the quantity of USP Cephapirin Sodium RS, in mg, used to prepare the *Standard preparation*;  $V_s$  is the final volume, in mL, of the *Standard preparation*;  $V_u$  is the entire volume of Intramammary Infusion, in mL, in one syringe; and  $r_u$  and  $r_s$  are the peak area and the average peak area of the cephapirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Cephapirin Sodium



$C_{17}H_{16}N_3NaO_6S_2$  445.45

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 3-[(acetyl-oxy)methyl]-8-oxo-7-[[[(4-pyridinylthio)acetyl]amino]-, monosodium salt, (6*R*-*trans*)-.

Monosodium (6*R*,7*R*)-3-(hydroxymethyl)-8-oxo-7-[2-(4-pyridylthio)acetamido]-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate acetate (ester) [24356-60-3].

» Cephapirin Sodium has a potency equivalent to not less than 855 µg and not more than 1000 µg of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ) per mg.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

### USP Reference standards (11)—

USP Cephapirin Sodium RS

USP Endotoxin RS

### Identification—

A: *Infrared Absorption* (197K).

B: It responds to the tests for *Sodium* (191).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 6.5 and 8.5, in a solution containing 10 mg of cephapirin per mL.

**Water Determination, Method I** (921): not more than 2.0%.

**Other requirements**—Where the label states that Cephapirin Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cephapirin for Injection*.

Where the label states that Cephapirin Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Cephapirin for Injection*.

### Assay—

*Solution A, Solution B, Mobile phase, Extraction solution, Dilution buffer, 10% Acetic acid solution, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Cephapirin Benzathine*.

**Assay preparation**—In duplicate, weigh about 50 mg of Cephapirin Sodium, and transfer into a 25-mL volumetric flask. Add about 2.5 mL of *Extraction solution* and 15.0 mL of *Dilution buffer*, and mix to dissolve. Add 7.0 mL of acetonitrile, and mix. Allow the flask to return to room temperature, and dilute with water to volume.

**Procedure**—Separately inject equal volumes (about 2 µL) of the duplicate *Standard preparation* and the duplicate *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in µg, of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ) in each mg of Cephapirin Sodium taken by the formula:

$$P(W_S / W_U)(V_U / V_S)(r_U / r_S)$$

in which *P* is the assigned potency, in µg of cephapirin per mg, of USP Cephapirin Sodium RS; *W<sub>S</sub>* and *W<sub>U</sub>* are the quantities of USP Cephapirin Sodium RS and Cephapirin Sodium, in mg, used to prepare the *Standard preparation* and the *Assay preparation*, respectively; *V<sub>U</sub>* and *V<sub>S</sub>* are the final volumes, in mL, of the *Assay preparation* and the *Standard*

*preparation*, respectively; and *r<sub>U</sub>* and *r<sub>S</sub>* are the average peak areas of the cephapirin peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephapirin Sodium Intramammary Infusion

» Cephapirin Sodium Intramammary Infusion is a suspension of Cephapirin Sodium in a suitable vegetable oil vehicle. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled quantity of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ). It contains a suitable dispersing agent.

**Packaging and storage**—Preserve in well-closed unit-dose disposable syringes at controlled room temperature.

**Labeling**—Label it to indicate that it is for veterinary use only.

### USP Reference standards (11)—

USP Cephapirin Sodium RS

**Identification, Infrared Absorption** (197K)—Prepare the test specimen as follows. Transfer the contents of 1 syringe of Intramammary Infusion to a 50-mL centrifuge tube, add 25 mL of toluene, mix for about 1 minute, and centrifuge. Remove and discard the toluene layer without disturbing the residue in the centrifuge tube. Wash the residue with two 25-mL portions of toluene. Dry the residue in vacuum at 60°, and use the dried residue as the test specimen. Mix the dried residue with 9 parts of potassium bromide, and record the IR spectrum, using the diffuse reflectance technique: the IR absorption spectrum so obtained corresponds to that of a similar dispersion of USP Cephapirin Sodium RS in potassium bromide.

**Water Determination, Method I** (921): not more than 1.0%, 10 mL of Intramammary Infusion being tested.

### Assay—

*Solution A, Solution B, Mobile phase, Extraction solution, Dilution buffer, 10% Acetic acid solution, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay under *Cephapirin Benzathine*.

**Assay preparation**—Express the entire contents of a syringe of the Intramammary Infusion into a centrifuge tube. For each mL of Intramammary Infusion, add 1.0 *n*-heptane and 1.0 mL of *Extraction solution*, cap, and mix on a vortex mixer at high speed for 5 minutes. Centrifuge for 5 minutes at a speed sufficient to break the emulsion. Remove the aqueous layer, and pass through a 0.45-µm nylon filter, discarding the first 0.5 mL. Transfer 2.5 mL of the filtered aqueous phase into a 25-mL volumetric flask that contains a solution composed of 15.0 mL of *Dilution buffer* and 7.0 mL of acetonitrile. Add water to volume, and mix well to obtain a single phase.

**Procedure**—Separately inject equal volumes (about 2 µL) of the duplicate *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the quantity, in mg, of cephapirin ( $C_{17}H_{17}N_3O_6S_2$ ) in each syringe of Intramammary Infusion taken by the formula:

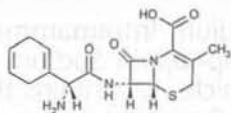
$$10PW(V_U / V_S)(r_U / r_S)$$

in which *P* is the assigned potency, in µg of cephapirin per mg, of USP Cephapirin Sodium RS; *W* is the quantity of USP Cephapirin Sodium RS, in mg, used to prepare the *Standard preparation*; *V<sub>S</sub>* is the final volume, in mL, of the *Standard preparation*; *V<sub>U</sub>* is the entire volume of Intramammary Infu-



sion, in mL, in one syringe; and  $r_U$  and  $r_S$  are the peak area and the average peak area of the cephradine peaks obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephadrine



$C_{16}H_{19}N_3O_4S$  349.40

5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[(amino-1,4-cyclohexadien-1-ylacetyl)amino]-3-methyl-8-oxo-, [6R-[6 $\alpha$ ,7 $\beta$ (R\*)]]-

(6R,7R)-7-[(R)-2-Amino-2-(1,4-cyclohexadien-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [38821-53-3].

Monohydrate 367.43 [31828-50-9(non-stoichiometric hydrate)].

Dihydrate 385.44 [58456-86-3].

» Cephadrine has a potency of not less than 900  $\mu$ g and not more than 1050  $\mu$ g of total cephalosporins per mg, calculated as the sum of cephradine ( $C_{16}H_{19}N_3O_4S$ ) and cephalixin ( $C_{16}H_{17}N_3O_4S$ ), calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is the dihydrate form, the label so indicates. Where the quantity of cephradine is indicated in the labeling of any preparation containing Cephadrine, this shall be understood to be in terms of anhydrous cephradine ( $C_{16}H_{19}N_3O_4S$ ). Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**USP Reference standards** (11)—

USP Cephadrine RS

USP Cephalixin RS

USP Endotoxin RS

**Identification**, *Infrared Absorption* (197K).

**Crystallinity** (695): meets the requirements.

**pH** (791): between 3.5 and 6.0, in a solution containing 10 mg per mL.

**Water Determination**, *Method I* (921): not more than 6.0%, except that if it is the dihydrate form, the limit is between 8.5% and 10.5%.

**Limit of cephalixin**—Using the chromatogram of the *Assay preparation* obtained in the *Assay*, calculate the percentage of cephalixin ( $C_{16}H_{17}N_3O_4S$ ) in the portion of Cephadrine taken by the formula:

$$100(r_{UX} / r_U)$$

in which  $r_{UX}$  is the cephalixin peak response in the chromatogram obtained from the *Assay preparation*, and  $r_U$  is the sum of the cephalixin and cephradine peak responses in the chromatogram obtained from the *Assay preparation*: not more than 5.0%, calculated on the anhydrous basis, is found.

**Other requirements**—Where the label states that Cephadrine is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Cephadrine for Injection*. Where the label states that Cephadrine must be subjected to further processing during the preparation of injectable dosage

forms, it meets the requirements for *Bacterial endotoxins* under *Cephadrine for Injection*.

### Assay—

**Mobile phase**—Prepare a mixture of water, methanol, 0.5 M sodium acetate, and 0.7 N acetic acid (782:200:15:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Filter the solution through a filter of 1  $\mu$ m or finer porosity, and degas before use.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cephadrine RS quantitatively in *Mobile phase* to obtain a solution having a known concentration of about 0.5 mg per mL.

**Resolution solution**—Prepare a solution in *Mobile phase* containing in each mL about 0.5 mg of USP Cephadrine RS and 0.5 mg of USP Cephalixin RS.

**Assay preparation**—Transfer about 50 mg of Cephadrine, accurately weighed, to a 100-mL volumetric flask, add about 30 mL of *Mobile phase*, and sonicate. Dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.8 for cephalixin and 1.0 for cephradine; and the resolution,  $R$ , between the cephalixin peak and the cephradine peak is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation*, and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu$ g, of total cephalosporins (sum of cephradine and cephalixin) in each mg of the Cephadrine taken by the formula:

$$100(CP / M)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cephadrine RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g per mg, of USP Cephadrine RS; M is the quantity, in mg, of Cephadrine taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the sums of the cephradine and cephalixin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephadrine Capsules

» Cephadrine Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cephradine, calculated as the sum of cephradine ( $C_{16}H_{19}N_3O_4S$ ) and cephalixin ( $C_{16}H_{17}N_3O_4S$ ).

**Packaging and storage**—Preserve in tight containers.

**Labeling**—The quantity of cephradine stated in the labeling is in terms of anhydrous cephradine ( $C_{16}H_{19}N_3O_4S$ ).

**USP Reference standards** (11)—

USP Cephadrine RS

USP Cephalixin RS

**Identification**—Mix the contents of 1 Capsule with water to obtain a solution having a concentration of about 3 mg of cephradine per mL, and filter (*test solution*). Place a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of binder-free silica gel



in a chamber containing a mixture of *n*-hexane and tetradecane (95:5) to a depth of about 1 cm, allow the solvent front to move the length of the plate, remove the plate from the chamber, and allow the solvent to evaporate. On this plate apply 10  $\mu$ L each of the *test solution* and a *Standard solution* containing 3 mg of USP Cephadrine RS per mL. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of 0.1 M citric acid, 0.1 M dibasic sodium phosphate, and a 1 in 15 solution of ninhydrin in acetone (60:40:1.5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, dry the plate for 10 minutes at 110°, and examine the chromatogram: the  $R_f$  value of the principal spot obtained from the *test solution* corresponds to that obtained from the *Standard solution*.

#### Dissolution (711)—

*Medium:* 0.12 N hydrochloric acid; 900 mL.

*Apparatus 1:* 100 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $C_{16}H_{19}N_3O_4S$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 255 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Cephadrine RS in the same medium.

*Tolerances*—Not less than 75% (Q) of the labeled amount of  $C_{16}H_{19}N_3O_4S$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, of the mixed contents of 4 Capsules in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: the Capsule contents lose not more than 7.0% of their weight.

#### Assay—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under *Cephadrine*.

*Assay preparation*—Transfer, as completely as possible, the contents of not fewer than 20 Capsules to a suitable tared container, determine the average weight per Capsule, and mix the combined contents. Transfer an accurately weighed portion of the powder, equivalent to about 125 mg of cephadrine, to a 250-mL volumetric flask, add 50 mL of *Mobile phase*, sonicate for about 15 minutes, and shake by mechanical means for about 10 minutes. Dilute with *Mobile phase* to volume, and mix. Filter a portion of this mixture through a filter having a porosity of 0.5  $\mu$ m or finer, discarding the first 5 mL of the filtrate. Use the filtrate as the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard cephadrine preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephadrine (sum of cephadrine and cephalixin) in the portion of Capsules taken by the formula:

$$0.25CP(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cephadrine RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g per mg, of USP Cephadrine RS; and  $r_U$  and  $r_S$  are the sums of the cephadrine and cephalixin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephadrine for Injection

» Cephadrine for Injection contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of cephadrine, calculated as the sum of cephadrine ( $C_{16}H_{19}N_3O_4S$ ) and cephalixin ( $C_{16}H_{17}N_3O_4S$ ).

#### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

#### USP Reference standards (11)—

USP Cephadrine RS

USP Cephalixin RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

**Identification**—Dilute the contents of 1 container of Cephadrine for Injection with water to obtain a test solution containing about 3 mg of cephadrine per mL. Proceed as directed in the *Identification test under Cephadrine Capsules*, beginning with "Place a suitable thin-layer chromatographic plate": the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the *Standard solution*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.20 USP Endotoxin Unit per mg of cephadrine.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 8.0 and 9.6, in a solution containing 10 mg per mL.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 5.0% of its weight.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements for *Uniformity of Dosage Units* (905) and *Labeling* (7), *Labels and Labeling for Injectable Products*.

#### Assay—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay under *Cephadrine*.

*Assay preparation 1* (where it is represented as being in a single-dose container)—Constitute Cephadrine for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and dilute quantitatively with *Mobile phase* to obtain a solution containing about 0.5 mg of cephadrine per mL.

*Assay preparation 2* (where the label states the quantity of cephadrine in a given volume of constituted solution)—Constitute Cephadrine for Injection in a volume of water, accurately measured, corresponding to the volume of solvent specified in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with *Mobile phase* to obtain a solution containing about 0.5 mg of cephadrine per mL.

*Procedure*—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and *Assay preparation 1* or *Assay preparation 2* into the chromatograph, record the chromato-



grams, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephradine (sum of cephradine and cephalixin) withdrawn from the container, or in the portion of constituted solution taken by the formula:

$$(CP)(L / 1000D)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Cephradine RS in the *Standard preparation*;  $P$  is the designated potency, in  $\mu\text{g}$  per mg, of USP Cephradine RS;  $L$  is the labeled quantity, in mg of cephradine, in the container taken to prepare *Assay preparation 1*, or in the volume of constituted solution taken to prepare *Assay preparation 2*;  $D$  is the concentration, in mg of cephradine per mL, of *Assay preparation 1* or *Assay preparation 2*, based on the labeled quantity in the container or in the portion of constituted solution taken, respectively, and the extent of dilution; and  $r_U$  and  $r_S$  are the sums of the cephradine and cephalixin peak responses obtained from *Assay preparation 1* or *Assay preparation 2* and the *Standard preparation*, respectively.

## Cephradine for Oral Suspension

» Cephradine for Oral Suspension is a dry mixture of Cephradine and one or more suitable buffers, colors, diluents, and flavors. It contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of cephradine, calculated as the sum of cephradine ( $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$ ) and cephalixin ( $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4\text{S}$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cephradine RS

USP Cephalixin RS

**Identification**—Constitute 1 container of Cephradine for Oral Suspension as directed in the labeling. Mix a portion of the resulting suspension with water to obtain a concentration of about 3 mg of cephradine per mL, and filter (*test solution*). Proceed as directed in the *Identification* test under *Cephradine Capsules*, beginning with "Place a suitable thin-layer chromatographic plate": the  $R_f$  value of the principal spot obtained from the *test solution* corresponds to that obtained from the *Standard solution*.

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 3.5 and 6.0, in the suspension constituted as directed in the labeling.

**Water Determination, Method I** (921): not more than 1.5%.

**Assay**—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Cephradine*.

*Assay preparation*—Constitute Cephradine for Oral Suspension as directed in the labeling. Dilute an accurately measured volume of the suspension so obtained, freshly mixed and free from air bubbles, quantitatively with *Mobile phase* to obtain a solution containing about 0.5 mg of cephradine per mL. Filter a portion of this mixture through a filter having a porosity of 0.5  $\mu\text{m}$  or finer, discarding the first 5 mL of the filtrate. Use the filtrate as the *Assay preparation*.

*Procedure*—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into

the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephradine (sum of cephradine and cephalixin) in each mL of constituted Oral Suspension taken by the formula:

$$(CP)(L / 1000D)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Cephradine RS in the *Standard preparation*;  $P$  is the designated potency, in  $\mu\text{g}$  per mg, of USP Cephradine RS;  $L$  is the labeled quantity, in mg of cephradine, in each mL of the constituted Oral Suspension;  $D$  is the concentration, in mg of cephradine per mL, of the *Assay preparation*, based on the labeled quantity of cephradine per mL of constituted Oral Suspension and the extent of dilution; and  $r_U$  and  $r_S$  are the sums of the cephradine and cephalixin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cephradine Tablets

» Cephradine Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cephradine, calculated as the sum of cephradine ( $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$ ) and cephalixin ( $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4\text{S}$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cephradine RS

USP Cephalixin RS

**Identification**—Mix a quantity of finely powdered Tablets with water to obtain a concentration of about 3 mg of cephradine per mL, and filter (*test solution*). Proceed as directed in the *Identification* test under *Cephradine Capsules*, beginning with "Place a suitable thin-layer chromatographic plate": the  $R_f$  value of the principal spot obtained from the *test solution* corresponds to that obtained from the *Standard solution*.

**Dissolution** (711)—

*Medium*: 0.12 N hydrochloric acid; 900 mL.

*Apparatus 2*: 75 rpm.

*Time*: 60 minutes.

*Procedure*—Determine the amount of  $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 255 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a *Standard solution* having a known concentration of USP Cephradine RS in the same medium.

*Tolerances*—Not less than 85% (Q) of the labeled amount of  $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water Determination, Method I** (921): not more than 6.0%.

**Assay**—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Cephradine*.

*Assay preparation*—Place not less than 5 Tablets in a high-speed glass blender jar containing an accurately measured volume of water, sufficient to yield a concentration of not less than 5 mg of cephradine per mL, and blend for  $4 \pm 1$  minutes. Dilute an accurately measured volume of this stock solution quantitatively and stepwise with *Mobile phase*



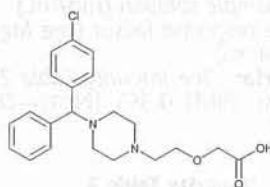
to obtain an *Assay preparation* containing about 0.5 mg of cephadrine per mL.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cephadrine (sum of cephadrine and cephalixin) in each Tablet taken by the formula:

$$(CP)(L/1000D)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cephadrine RS in the *Standard preparation*; P is the designated potency, in  $\mu$ g per mg, of USP Cephadrine RS; L is the labeled quantity, in mg of cephadrine, in each Tablet; D is the concentration, in mg of cephadrine per mL, of the *Assay preparation*, based on the labeled quantity per Tablet, the number of Tablets taken, and the extent of dilution; and  $r_U$  and  $r_S$  are the sums of the cephadrine and cephalixin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cetirizine Hydrochloride



$C_{21}H_{25}ClN_2O_3 \cdot 2HCl$  461.81  
 ( $\pm$ )-[2-[4-[(4-Chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid, dihydrochloride;  
 ( $\pm$ )-[2-[4-(p-Chloro- $\alpha$ -phenylbenzyl)-1-piperazinyl]ethoxy]acetic acid, dihydrochloride [83881-52-1].

### DEFINITION

Cetirizine Hydrochloride contains NLT 98.0% and NMT 102.0% of  $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ , calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TEST—GENERAL, Chloride** (191): Meets the requirements

### ASSAY

#### PROCEDURE

**Mobile phase:** Acetonitrile, water, and 1 M sulfuric acid (93:6.6:0.4)

**Standard solution:** 0.5 mg/mL USP Cetirizine Hydrochloride RS in *Mobile phase*

**Sample solution:** 0.5 mg/mL Cetirizine Hydrochloride in *Mobile phase*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L3

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$  in the portion of Cetirizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cetirizine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

#### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.2%

#### Delete the following:

- **HEAVY METALS, Method I** (231): 10 ppm (Official 1-Jan-2018)

#### Organic Impurities

[NOTE—It is recommended that *Test 2* be performed if either cetirizine ethanol (2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol) or cetirizine acetic acid (2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid) may be present in the test substance.]

#### PROCEDURE 1

**Mobile phase and Sample solution:** Proceed as directed in the *Assay*.

**System suitability solution:** 4  $\mu$ g/mL each of USP Cetirizine Hydrochloride RS and USP Cetirizine Related Compound A RS in *Mobile phase*

**Standard solution:** 0.5  $\mu$ g/mL of USP Cetirizine Hydrochloride RS in *Mobile phase*

**Chromatographic system:** Prepare as directed in the *Assay*.

(See *Chromatography* (621), *System Suitability*.)

**Run time:** Three times the retention time of cetirizine

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0 for cetirizine, *System suitability solution*

**Resolution:** NLT 2.0 between cetirizine and cetirizine related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0% cetirizine, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cetirizine hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response for cetirizine from the *Standard solution*



- $C_s$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_u$  = concentration of Cetirizine Hydrochloride in the *Sample solution* (mg/mL)  
 $F$  = relative response factor (see *Impurity Table 1* for values)

Acceptance criteria: See *Impurity Table 1*.

Total impurities: NMT 0.3%. [NOTE—Disregard peaks below 0.02%.]

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
4-CBH <sup>a</sup>	0.3	1.4	0.1
Dimer <sup>b</sup>	0.5	1.8	0.1
2-Chlorocetirizine <sup>c</sup>	0.85	0.49	0.1
Cetirizine related compound A <sup>d</sup>	0.9	0.95	0.1
Cetirizine	1.0	—	—
Deschlorocetirizine <sup>e</sup>	1.4	0.45	0.1
CBHP <sup>f</sup>	1.45	1.6	0.1
Any individual unspecified impurity	—	1.0	0.1

<sup>a</sup> 4-Chlorobenzhydrol.

<sup>b</sup> 1,4-Bis[(4-chlorophenyl)phenylmethyl]piperazine.

<sup>c</sup> 2-[2-[4-[(2-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.

<sup>d</sup> 2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid, ethyl ester (cetirizine ethyl ester).

<sup>e</sup> 2-[2-[4-(Diphenylmethyl)piperazin-1-yl]ethoxy]acetic acid.

<sup>f</sup> 1-[(4-Chlorophenyl)phenylmethyl]piperazine.

#### • PROCEDURE 2

**Solution A:** 2 g/L tetrabutyl ammonium hydrogen sulfate and 3 g/L of monobasic sodium phosphate monohydrate in water. Adjust with 1 N sodium hydroxide to a pH of  $2.8 \pm 0.05$ .

**Solution B:** Methanol

**Buffer:** 1.4 g/L monobasic sodium phosphate monohydrate and 2.7 g/L of dibasic sodium phosphate heptahydrate. Adjust with either 1 N sodium hydroxide or 10% phosphoric acid to a pH of  $6.9 \pm 0.1$ .

**Diluent:** Acetonitrile and *Buffer* (1:1)

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Flow Rate (mL/min)
0	58	42	1.2
40	58	42	1.2
68	20	80	1.5
108	20	80	1.5
110	58	42	1.2
120	58	42	1.2

**Standard solution:** 2 µg/mL of USP Cetirizine Hydrochloride RS in *Diluent*

**Sample solution:** 2 mg/mL cetirizine hydrochloride in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 232 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Injection size: 10 µL

#### System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2

Column efficiency: NLT 6000 theoretical plates

Relative standard deviation: NMT 5.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cetirizine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response for each impurity from the *Sample solution*

$r_s$  = peak response for cetirizine from the *Standard solution*

$C_s$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Cetirizine Hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Impurity Table 2* for values)

Acceptance criteria: See *Impurity Table 2*.

Total impurities: NMT 0.3%. [NOTE—Disregard peaks below 0.05%.]

Impurity Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Deschlorocetirizine <sup>a</sup>	0.35	0.56	0.1
Cetirizine ethanol <sup>b</sup>	0.53	1.2	0.1
CBHP <sup>c</sup>	0.66	1.3	0.1
2-Chlorocetirizine <sup>d</sup>	0.70	0.52	0.1
Cetirizine methyl ester <sup>e</sup>	0.81	0.96	0.1
3-Chlorocetirizine <sup>f</sup>	0.87	0.52	0.1
Cetirizine	1.0	—	—
Cetirizine acetic acid <sup>g</sup>	1.15	0.97	0.1
Cetirizine N-oxide <sup>h</sup>	1.25	0.81	0.1
4-CBH <sup>i</sup>	1.55	1.2	0.1
4-Chlorobenzophenone <sup>j</sup>	1.66	0.50	0.1

<sup>a</sup> 2-[2-[4-(Diphenylmethyl)piperazin-1-yl]ethoxy]acetic acid.

<sup>b</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.

<sup>c</sup> 1-[(4-Chlorophenyl)phenylmethyl]piperazine.

<sup>d</sup> 2-[2-[4-[(2-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.

<sup>e</sup> Methyl 2-[2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetate.

<sup>f</sup> 2-[2-[4-[(3-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.

<sup>g</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid.

<sup>h</sup> 2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid N-oxide.

<sup>i</sup> 4-Chlorobenzhydrol.

<sup>j</sup> (4-Chlorophenyl)phenylmethanone.

<sup>k</sup> 1,4-Bis[(4-chlorophenyl)phenylmethyl]piperazine.



Impurity Table 2 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cetirizine dimer <sup>a</sup>	2.48	1.4	0.1
Any individual unspecified impurity	—	1.0	0.10

<sup>a</sup> 2-[2-[4-(Diphenylmethyl)piperazin-1-yl]ethoxy]acetic acid.<sup>b</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.<sup>c</sup> 1-[(4-Chlorophenyl)phenylmethyl]piperazine.<sup>d</sup> 2-[2-[4-[(2-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.<sup>e</sup> Methyl 2-[2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetate.<sup>f</sup> 2-[2-[4-[(3-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.<sup>g</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid.<sup>h</sup> 2-[2-[4-[(4-Chlorophenyl)(phenyl)methyl]piperazin-1-yl]ethoxy]acetic acid N<sup>1</sup>-oxide.<sup>i</sup> 4-Chlorobenzhydrol.<sup>j</sup> (4-Chlorophenyl)phenylmethanone.<sup>k</sup> 1,4-Bis[(4-chlorophenyl)phenylmethyl]piperazine.**SPECIFIC TESTS**

- **pH (791):** 1.2–1.8, in an aqueous solution 1 in 20
- **LOSS ON DRYING (731):** Dry a sample at 105° to a constant weight: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light and moisture. Store at room temperature.
- **LABELING:** Label it to indicate with which impurity procedures the article complies.
- **USP REFERENCE STANDARDS (11)**
  - USP Cetirizine Hydrochloride RS
  - USP Cetirizine Related Compound A RS
  - (RS)-2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid ethyl ester dihydrochloride.
  - C<sub>23</sub>H<sub>29</sub>ClN<sub>2</sub>O<sub>3</sub> · 2HCl 489.86

**Cetirizine Hydrochloride Oral Solution****DEFINITION**

Cetirizine Hydrochloride Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of C<sub>21</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>3</sub> · 2HCl.

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

**ASSAY**• **PROCEDURE**

Solution A: Acetonitrile

Solution B: 1.36 g/L of monobasic potassium phosphate in water. Adjust with a 2% solution of phosphoric acid in water to a pH of 3.5 ± 0.05.

Diluent: Acetonitrile and water (3:7)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	5	95
15	5	95
22	25	75
35	25	75
40	5	95
50	5	95

**Standard stock solution:** 5 mg/mL of USP Cetirizine Hydrochloride RS in water

**Standard solution:** 0.1 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*, from the *Standard stock solution*

**Sample solution:** Transfer an amount of Oral Solution to a suitable volumetric flask to obtain a nominal concentration of 0.1 mg/mL of cetirizine hydrochloride. Dissolve in 60% of the flask volume of *Diluent* by swirling. Sonicate 3 min, and dilute with *Diluent* to volume. Pass through a suitable filter.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 233 nm

Column: 4.6-mm × 25-cm; 5-μm packing L10

Column temperature: 50°

Flow rate: 2 mL/min

Injection size: 20 μL

**System suitability**Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of C<sub>21</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>3</sub> · 2HCl in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r<sub>U</sub> = peak response from the *Sample solution*r<sub>S</sub> = peak response from the *Standard solution*C<sub>S</sub> = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)C<sub>U</sub> = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**

- **DELIVERABLE VOLUME (698):** Meets the requirements

**IMPURITIES****Organic Impurities**• **PROCEDURE**

**Solution A:** Transfer 50 mL of water to a 100-mL volumetric flask, add 5.5 mL of sulfuric acid, and dilute with water to volume.

**Mobile phase:** Acetonitrile, water, and *Solution A* (965:33:1)

**Diluent:** Acetonitrile and water (7:13)

**Standard solution:** 6 μg/mL of USP Cetirizine Hydrochloride RS in *Diluent*

**Sample solution:** 0.6 mg/mL of cetirizine hydrochloride in *Diluent*. Transfer an amount of Oral Solution to a suitable volumetric flask, dissolve in *Diluent*, sonicate for 10 min, and dilute with *Diluent* to volume. Pass through a suitable filter.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-μm packing L3

Column temperature: 30°

Flow rate: 2 mL/min

Injection size: 10 μL

**System suitability**Sample: *Standard solution***Suitability requirements**

Column efficiency: NLT 10,000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 5.0%

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response for each impurity from the *Sample solution* $r_S$  = peak response for cetirizine from the *Standard solution* $C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)Acceptance criteria: See *Impurity Table 1*.

Total impurities: NMT 0.8%

**Impurity Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cetirizine acetic acid <sup>a</sup>	0.69	P <sup>b</sup>
2-Chlorocetirizine <sup>c</sup>	0.83	P
Cetirizine	1.00	—
Cetirizineethanol <sup>d</sup>	1.30	P
Ethoxycetirizine <sup>e</sup>	1.38	P
CBHP <sup>f</sup>	1.52	P
Propylene glycol ester of cetirizine (diastereomer 1) <sup>g</sup>	1.53	0.2
Propylene glycol ester of cetirizine (diastereomer 2) <sup>g</sup>	1.61	0.2
Deschlorocetirizine <sup>h</sup>	1.65	P
Glyceryl ester of cetirizine <sup>i</sup>	2.20	0.5
Any individual unspecified impurity	—	0.2

<sup>a</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid.<sup>b</sup> P = Process impurity. Provided for information only; the content is not calculated and not reported. The content is controlled in the drug substance monograph.<sup>c</sup> 2-[2-[4-[(2-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy]acetic acid.<sup>d</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.<sup>e</sup> 2-[2-[2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy] ethoxy]acetic acid (ethoxycetirizine).<sup>f</sup> 1-[(4-Chlorophenyl)phenylmethyl]piperazine.<sup>g</sup> 2-Hydroxypropyl 2-(2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy)acetate.<sup>h</sup> 2-[2-[4-(Diphenylmethyl)piperazin-1-yl]ethoxy]acetic acid.<sup>i</sup> 2,3-Dihydroxypropyl 2-(2-[4-[(4-chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy)acetate.**SPECIFIC TESTS**• **PH (791):** 4.0–5.1• **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed 100 cfu/mL, and the total combined molds and yeasts count does not exceed 10 cfu/mL. It meets the requirements of the tests for absence of *Escherichia coli*.**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and protect from light. Store at controlled room temperature or in a cold place.• **USP REFERENCE STANDARDS (11)**

USP Cetirizine Hydrochloride RS

**Cetirizine Hydrochloride Tablets****DEFINITION**Cetirizine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ).**IDENTIFICATION**• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.**ASSAY**• **PROCEDURE****Solution A:** 2 N sulfuric acid and water (2:33)**Buffer:** 2.9 mL/L of phosphoric acid in water**Mobile phase:** Acetonitrile and *Buffer* (3:7)**Diluent:** Acetonitrile, *Solution A*, and water (100:1:100)**Standard solution:** 0.2 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent***Sample solution:** 0.2 mg/mL of cetirizine hydrochloride in *Diluent* from NLT 20 powdered Tablets. [NOTE—Sonicate, if necessary.]**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 10 μL

Run time: 1.3 times the retention time of cetirizine

**System suitability**Sample: *Standard solution***Suitability requirements**

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)



Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

#### Test 1

Medium: Water; 900 mL, degassed

Apparatus 2: 50 rpm

Time: 30 min

Buffer: 2.9 mL/L of phosphoric acid in water

Mobile phase: Acetonitrile and Buffer (2:3)

Standard solution: 11 µg/mL of USP Cetirizine Hydrochloride RS in water. This solution can be stored for 48 h at room temperature.

Sample solution: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 50 µL

Run time: 1.3 times the retention time of cetirizine

#### System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the Standard solution (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of Medium, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) is dissolved.

Test 2: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 30 min

Buffer: 0.4 g/L of 1-heptane sulfonic acid sodium salt  
Mobile phase: Acetonitrile and Buffer (50:50). Adjust with 0.1 N sulfuric acid to a pH of 3.5.

Standard solution: 11 µg/mL of USP Cetirizine Hydrochloride RS in Medium

Sample solution: Pass a 20-mL portion of the solution under test through a nylon filter of 0.45-µm pore size. Discard the first 10 mL of the filtrate.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 210 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 1.5 mL/min

Injection volume: 50 µL

Run time: 1.6 times the retention time of cetirizine

#### System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the Standard solution (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of Medium, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) is dissolved.

Test 3: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 3.

Medium: Water; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: ( $L/900$ ) mg/mL of USP Cetirizine Hydrochloride RS in water, where  $L$  is the label claim of cetirizine hydrochloride, in mg/Tablet

Sample solution: Centrifuge a portion of the solution under test for NLT 15 min at 3000 rpm.

#### Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: UV

Analytical wavelength: UV 231 nm

Blank: Medium

Path length: 1 cm

#### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the Sample solution

$A_S$  = absorbance of the Standard solution

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the Standard solution (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of Medium, 900 mL

Tolerances: NLT 80% (Q) of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

Solution A: 2 N sulfuric acid and water (2:33)

Buffer: 3.4 g/L of tetrabutyl ammonium hydrogen sulfate in water

Diluent: Acetonitrile, Solution A, and water (910:27:63)

Mobile phase: Acetonitrile, Solution A, and Buffer (93:5:2)

Standard solution: 1.5 µg/mL of USP Cetirizine Hydrochloride RS in Diluent

Sample solution: 0.5 mg/mL of cetirizine hydrochloride in Diluent from NLT 20 powdered Tablets. [NOTE—Sonicate, if necessary.]



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm

Column: 4.0-mm × 25-cm; 5-μm packing L3

Flow rate: 0.8 mL/min

Injection volume: 20 μL

Run time: 2.5 times the retention time of cetirizine

**System suitability**Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 10.0%

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution* $r_S$  = peak response of cetirizine from the *Standard solution* $C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL) $F$  = relative response factor (see *Table 1*)Acceptance criteria: See *Table 1*.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cetirizine lactose ester <sup>a</sup>	0.56	1.0	0.5
Cetirizine	1.0	—	—
Cetirizine ethanol <sup>b</sup>	1.67	1.2	0.4
Any unspecified degradation product	—	—	0.2
Total impurities	—	—	1

<sup>a</sup> 6-O-[2-(2-{4-[(4-Chlorophenyl)(phenyl)methyl]piperazin-1-yl}ethoxy)acetyl]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose.<sup>b</sup> 2-[4-[(4-Chlorophenyl)(phenyl)methyl]piperazin-1-yl]ethanol.**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers, and store below 30°.

- LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.

- USP REFERENCE STANDARDS (11)**  
USP Cetirizine Hydrochloride RS

## Cetirizine Hydrochloride Orally Disintegrating Tablets

**DEFINITION**Cetirizine Hydrochloride Orally Disintegrating Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cetirizine hydrochloride (C<sub>21</sub>H<sub>25</sub>ClN<sub>2</sub>O<sub>3</sub> · 2HCl).**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE**

**Buffer A:** Dissolve 13.2 g of sodium 1-decanesulfonate in 2760 mL of water. Add 5.5 g of monobasic sodium phosphate and 1.2 mL of phosphoric acid. Pass through a suitable filter.

**Buffer B:** Dissolve 13.2 g of sodium 1-decanesulfonate in 2000 mL of water. Add 5.5 g of monobasic sodium phosphate and 1.2 mL of phosphoric acid. Pass through a suitable filter.

**Solution A:** Acetonitrile and *Buffer A* (31:69)**Solution B:** Acetonitrile and *Buffer B* (50:50)**Mobile phase:** See *Table 1*.**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
35.0	0	100
35.1	100	0
45.0	100	0

**Diluent:** Acetonitrile and 0.01 N hydrochloric acid (20:80)

**System suitability stock solution:** 0.04 mg/mL of USP Meclizine Related Compound A RS, prepared as follows. Transfer a quantity of the compound into a suitable volumetric flask. Add 5% of the flask volume of acetonitrile to dissolve the components. Sonicate if needed. Dilute with *Diluent* to volume.

**System suitability solution:** 0.4 mg/mL of USP Cetirizine Hydrochloride RS and 1.2 μg/mL of USP Meclizine Related Compound A RS, prepared as follows. In a 100-mL volumetric flask, dissolve 40 mg of USP Cetirizine Hydrochloride RS in *Diluent*, and then pipette 3.0 mL of the *System suitability stock solution*. Dilute with *Diluent* to volume.

**Standard solution:** 0.4 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*

**Sample solution:** Nominally 0.4 mg/mL of cetirizine hydrochloride, prepared as follows. Transfer 20 Tablets into a 500-mL volumetric flask, add 375 mL of *Diluent*, and stir for at least 30 min. Dilute with *Diluent* to volume and pass a portion of this solution through a suitable filter (GHP or PVDF), discarding the first 1 mL of filtrate.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 230 nm. For *Identification test B*, use a diode array detector in the range of 210–300 nm.

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

**System suitability**Samples: *System suitability solution* and *Standard solution*[NOTE—Identify the components on the basis of their relative retention times, as shown in *Table 2*.]**Suitability requirements**

**Resolution:** NLT 1.5 between cetirizine and meclizine related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*  
 $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**

• **DISINTEGRATION** (701): NMT 30 s

• **DISSOLUTION** (711)

**Medium:** pH 6.5 phosphate buffer (4.77 g/L monobasic potassium phosphate and 2.62 g/L of dibasic potassium phosphate); 900 mL, degassed by USP procedure

**Apparatus 2:** 50 rpm

**Time:** 15 min

**Buffer:** 2.7 g/L of monobasic potassium phosphate in water. Add 0.6 mL/L of phosphoric acid.

**Mobile phase:** Acetonitrile and *Buffer* (33:67)

**Diluent:** Acetonitrile and 0.01 N hydrochloric acid (20:80)

**Standard stock solution:** 0.1 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*

**Standard solution:** 0.01 mg/mL of USP Cetirizine Hydrochloride RS from *Standard stock solution*, in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size, discarding the first 1–2 mL.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm  $\times$  7.5-cm; 3.5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 2.5 mL/min

**Injection volume:** 100  $\mu$ L

**Run time:** 2.7 times the retention time of cetirizine

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times C_s \times 1/L \times V \times 100$$

$r_u$  = peak response from the *Sample solution*  
 $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)  
 $L$  = label claim (mg/Tablet)  
 $V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) is dissolved.

• **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

**IMPURITIES**• **ORGANIC IMPURITIES**

**Buffer A, Buffer B, Solution A, Solution B, Mobile phase, and Diluent:** Proceed as directed in the Assay.  
**System suitability stock solution:** 0.04 mg/mL each of USP Cetirizine Related Compound G RS, USP Cetirizine

Related Compound B RS, USP 4-Chlorobenzophenone RS, USP Meclizine Related Compound A RS, and USP Cetirizine Related Compound A RS, and 0.06 mg/mL each of USP Hydroxyzine Related Compound A RS and USP Cetirizine Related Compound C RS, prepared as follows. Transfer a quantity of each compound into a suitable volumetric flask. Add 5% of the flask volume of acetonitrile to dissolve the compounds. Sonicate as needed. Dilute with *Diluent* to volume.

**System suitability solution:** 0.4 mg/mL of USP Cetirizine Hydrochloride RS; 1.2  $\mu$ g/mL each of USP Cetirizine Related Compound G RS, USP Cetirizine Related Compound B RS, USP 4-Chlorobenzophenone RS, USP Meclizine Related Compound A RS, and USP Cetirizine Related Compound A RS; and 1.8  $\mu$ g/mL each of USP Hydroxyzine Related Compound A RS and USP Cetirizine Related Compound C RS, prepared as follows. In a 100-mL volumetric flask, dissolve 40 mg of USP Cetirizine Hydrochloride RS in *Diluent*, and then pipette 3.0 mL of the *System suitability stock solution*. Dilute with *Diluent* to volume.

**Standard stock solution:** 0.04 mg/mL each of USP Cetirizine Hydrochloride RS, USP Cetirizine Related Compound G RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS, prepared as follows. Transfer a quantity of each compound into a suitable volumetric flask. Add 5% of the flask volume of acetonitrile to dissolve the compounds. Sonicate if needed. Dilute with *Diluent* to volume.

**Standard solution:** 0.8  $\mu$ g/mL each of USP Cetirizine Hydrochloride RS, USP Cetirizine Related Compound G RS, USP Hydroxyzine Related Compound A RS, and USP 4-Chlorobenzophenone RS from *Standard stock solution*, in *Diluent*

**Sample solution:** Nominally 400  $\mu$ g/mL of cetirizine hydrochloride, prepared as follows. Transfer 20 Tablets into a 500-mL volumetric flask, add 375 mL of *Diluent*, and stir for at least 30 min. Dilute with *Diluent* to volume and pass a portion of this solution through a suitable filter (GHP or PVDF), discarding the first 1 mL of filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm and 260 nm

**Column:** 4.6-mm  $\times$  15-cm; 3.5- $\mu$ m packing L1

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection volume:** 50  $\mu$ L

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between cetirizine and meclizine related compound A, *System suitability solution*

**Relative standard deviation:** NMT 10.0% for each component, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Chromatograph the *System suitability solution*, and identify the components on the basis of their relative retention times, as shown in Table 2. Use the peak response at 260 nm for 4-chlorobenzophenone. Use 230 nm for all other degradation products.  
Calculate the percentage of cetirizine related compound G, hydroxyzine related compound A, and 4-chlorobenzophenone in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of cetirizine related compound G, hydroxyzine related compound A, or 4-chlorobenzophenone from the *Sample solution*



- $r_s$  = peak response of the appropriate USP Reference Standard from the *Standard solution*
- $C_s$  = concentration of the appropriate USP Reference Standard in the *Standard solution* ( $\mu\text{g/mL}$ )
- $C_u$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* ( $\mu\text{g/mL}$ )
- Calculate the percentage of all other degradation products in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of any other degradation product from the *Sample solution*
- $r_s$  = peak response of cetirizine from the *Standard solution*
- $C_s$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* ( $\mu\text{g/mL}$ )
- $C_u$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* ( $\mu\text{g/mL}$ )
- Acceptance criteria: See Table 2. Disregard any peak less than 0.05%.

Table 2

Compound	Relative Retention Time	Wavelength (nm)	Acceptance Criteria, NMT (%)
Cetirizine related compound B	0.88	230	0.2
Mecizine related compound A	0.95	230	0.2
Cetirizine	1.00	230	—
Bromocetirizine	1.07	—	Pa
Cetirizine related compound C	1.09	—	Pa
Cetirizine related compound G	1.15	230	0.2
Hydroxyzine related compound A	1.23	230	0.2
4-Chlorobenzophenone	1.45	260	0.2
Cetirizine related compound A	1.53	230	0.2
Any individual unspecified degradation product	—	230	0.2
Total degradation products	—	—	0.8

\* Process impurity included in the table for identification only. Process impurities are controlled in the drug substance, and are not to be reported or included in the total impurities for the drug product.

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
  - USP Cetirizine Hydrochloride RS
  - USP Cetirizine Related Compound A RS  
2-(2-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy)acetic acid, ethyl ester dihydrochloride.  
 $\text{C}_{23}\text{H}_{29}\text{ClN}_2\text{O}_3 \cdot 2\text{HCl}$  489.86
  - USP Cetirizine Related Compound B RS  
2-(4-Benzhydrylpiperazin-1-yl)ethan-1-ol.  
 $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}$  296.41
  - USP Cetirizine Related Compound C RS  
2-(2-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethoxy)acetamide.

- $\text{C}_{21}\text{H}_{26}\text{ClN}_3\text{O}_2$  387.90  
USP Cetirizine Related Compound G RS  
2-{4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl}ethanol.  
 $\text{C}_{19}\text{H}_{23}\text{ClN}_2\text{O}$  330.85
- USP 4-Chlorobenzophenone RS  
(4-Chlorophenyl)phenylmethanone.  
 $\text{C}_{13}\text{H}_9\text{ClO}$  216.66
- USP Hydroxyzine Related Compound A RS  
1-[(4-Chlorophenyl)phenylmethyl]piperazine.  
 $\text{C}_{17}\text{H}_{19}\text{ClN}_2$  286.80
- USP Mecizine Related Compound A RS  
4-Chlorobenzhydrol.  
 $\text{C}_{13}\text{H}_{11}\text{ClO}$  218.68

## Cetirizine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets

### DEFINITION

Cetirizine Hydrochloride and Pseudoephedrine Hydrochloride Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cetirizine hydrochloride ( $\text{C}_{21}\text{H}_{25}\text{ClN}_2\text{O}_3 \cdot 2\text{HCl}$ ) and pseudoephedrine hydrochloride ( $\text{C}_{10}\text{H}_{15}\text{NO} \cdot \text{HCl}$ ).

### IDENTIFICATION

**A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### CETIRIZINE HYDROCHLORIDE

**Buffer:** 3.5 g/L of monobasic ammonium phosphate and 1.0 g/L of tetrabutylammonium bisulfate in water. Adjust with phosphoric acid to a pH of 2.5.

**Diluent:** Methanol and *Buffer* (2:3)

**Solution A:** Acetonitrile, methanol, and *Buffer* (9:2:29)

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
27.0	100	0
30.0	0	100
30.1	100	0
35.0	100	0

**Standard stock solution:** 0.5 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*. [NOTE—Sonicate to dissolve.]

**Standard solution:** 0.025 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent* from the *Standard stock solution*

**Sample solution:** 0.025 mg/mL of cetirizine hydrochloride (from NMT 10 finely powdered Tablets) prepared as follows. Dissolve the Tablets first in methanol, using 22.5% of the final flask volume. Sonicate for NLT 20 min with vigorous swirling every 5 min. To the solution add a volume of *Buffer* equal to 26% of the final flask volume. Allow the solution to equilibrate to room temperature. Dilute with *Diluent* to volume. Pass a portion through a membrane filter of 0.45- $\mu\text{m}$  pore size.

#### Chromatographic system

(See Chromatography (621), System Suitability.)



Mode: LC

Detector: UV 230 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing L1

Temperatures

Column: 30°

Autosampler: 5°

Flow rate: 1 mL/min

Injection volume: 25 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 3000 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cetirizine from the *Sample solution*

$r_S$  = peak response of cetirizine from the *Standard solution*

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### • PSEUDOEPHEDRINE HYDROCHLORIDE

Buffer: 0.8 g/L of ammonium acetate in water. To 1 L of the solution add 1.0 mL of triethylamine. Adjust with glacial acetic acid to a pH of 4.5.

Mobile phase: Acetonitrile and Buffer (3:7)

Standard solution: 0.5 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Mobile phase*. [NOTE—Sonicate to dissolve.]

Sample stock solution: 2.4 mg/mL of pseudoephedrine hydrochloride (from 5 finely powdered Tablets) prepared as follows. Dissolve the crushed Tablets first in acetonitrile, using 24% of the final flask volume. Sonicate for NLT 15 min. To the solution add a volume of Buffer equal to 56% of the final flask volume. Sonicate for NLT 15 min. Shake the flask for NLT 10 min. Allow the solution to equilibrate to room temperature. Dilute with *Mobile phase* to volume. Centrifuge a portion for 15 min to obtain a clear supernatant.

Sample solution: 0.5 mg/mL of pseudoephedrine hydrochloride in *Mobile phase* from the *Sample stock solution*. Pass the solution through a membrane filter of 0.45-μm pore size.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 5-μm packing L9

Flow rate: 1.5 mL/min

Injection volume: 25 μL

Run time: 2 times the retention time of pseudoephedrine

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 1000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of pseudoephedrine from the *Sample solution*

$r_S$  = peak response of pseudoephedrine from the *Standard solution*

$C_S$  = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of pseudoephedrine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

###### Test 1

Medium: 0.1 N hydrochloric acid; 500 mL, deaerated

Apparatus 1: 100 rpm

Time: 30 min for cetirizine hydrochloride and 30 min (used only for adjustments in the calculations); 1, 2, and 6 h for pseudoephedrine hydrochloride

Buffer: 0.77 g/L of ammonium acetate in water. To 1 L of the solution add 1.0 mL of triethylamine. Adjust with glacial acetic acid to a pH of  $4.5 \pm 0.05$ .

Mobile phase: Acetonitrile and Buffer (3:7)

Standard stock solution: 0.5 mg/mL of USP Cetirizine Hydrochloride RS in water

Standard solution: 0.24 mg/mL of USP Pseudoephedrine Hydrochloride RS and 0.01 mg/mL of USP Cetirizine Hydrochloride RS in *Medium* from the *Standard stock solution*

Sample solution: At the times specified, withdraw 5 mL of the solution under test, and pass through a suitable filter of 0.45-μm pore size, discarding the first few mL.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV, 230 nm for cetirizine hydrochloride, 254 nm for pseudoephedrine hydrochloride

Column: 4.6-mm × 15-cm; 5-μm packing L9

Flow rate: 1.5 mL/min

Injection volume: 25 μL

Run time: 2 times the retention time of pseudoephedrine

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0 for both cetirizine and pseudoephedrine

Relative standard deviation: NMT 2.0% for both cetirizine and pseudoephedrine

Analysis

Samples: *Standard solution* and *Sample solution*  
Calculate the percentage of cetirizine hydrochloride dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response of cetirizine from the *Sample solution*

$r_S$  = peak response of cetirizine from the *Standard solution*

$C_S$  = concentration of cetirizine hydrochloride in the *Standard solution* (mg/mL)

$L$  = label claim for cetirizine hydrochloride (mg/Tablet)

$V$  = volume of *Medium*, 500 mL



Calculate the percentage of pseudoephedrine hydrochloride dissolved at each time point:

$$Q_{30} = (r_u/r_s) \times (C_s/L) \times V \times 100$$

$$Q_1 = (Q_{30} \times 5/500) + [(r_u/r_s) \times (C_s/L) \times 495 \times 100]$$

$$Q_2 = (Q_{30} \times 5/500) + (Q_1 \times 5/495) + [(r_u/r_s) \times (C_s/L) \times 490 \times 100]$$

$$Q_6 = (Q_{30} \times 5/500) + (Q_1 \times 5/495) + (Q_2 \times 5/490) + [(r_u/r_s) \times (C_s/L) \times 485 \times 100]$$

$r_u$  = peak response of pseudoephedrine from the *Sample solution*

$r_s$  = peak response of pseudoephedrine from the *Standard solution*

$C_s$  = concentration of pseudoephedrine hydrochloride in the *Standard solution* (mg/mL)

$L$  = label claim for pseudoephedrine hydrochloride (mg/Tablet)

$V$  = initial volume of *Medium*, 500 mL

#### Tolerances

**Cetirizine hydrochloride:** NLT 80% (Q) of the labeled amount of cetirizine hydrochloride is dissolved in 30 min.

**Pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ):** See *Table 2*.

**Table 2**

Time (h)	Amount Dissolved
1	30%–50%
2	50%–70%
6	NLT 80%

The percentages of the labeled amount of pseudoephedrine hydrochloride dissolved at the times specified conform to *Acceptance Table 2* in (711).

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.1 N HCl; 500 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min for cetirizine hydrochloride and 30 min (used only for adjustments in the calculations); 1, 2, 4 and 8 h for pseudoephedrine hydrochloride

**Buffer:** 6.8 g/L of sodium acetate and 16.2 g/L of sodium 1-octanesulfonate

**Mobile phase:** Methanol and *Buffer* (50:50). Adjust with glacial acetic acid to a pH of 5.5.

**Standard solution:** 0.01 mg/mL of USP Cetirizine Hydrochloride RS and 0.24 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Medium*

**Sample solution:** Pass a 5-mL portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 242 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu$ m packing L1

**Column temperature:** 35°

**Flow rate:** 2 mL/min

**Injection volume:** 100  $\mu$ L

#### System suitability

[NOTE—The relative retention times for pseudoephedrine and cetirizine are 1.0 and 2.9, respectively.]

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 2000 theoretical plates for both pseudoephedrine and cetirizine

**Tailing factor:** NMT 2.0 for both pseudoephedrine and cetirizine

**Relative standard deviation:** NMT 2.0% for both pseudoephedrine and cetirizine

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) dissolved:

$$\text{Result} = (r_u/r_s) \times (C_s/L) \times V \times 100$$

$r_u$  = peak response of cetirizine from the *Sample solution*

$r_s$  = peak response of cetirizine from the *Standard solution*

$C_s$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of *Medium*, 500 mL

Calculate the concentration ( $C_i$ ) of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the sample withdrawn from the vessel at each time point ( $i$ ) shown in *Table 3*:

$$\text{Result}_i = (r_u/r_s) \times C_s$$

$r_u$  = peak response of pseudoephedrine from the *Sample solution*

$r_s$  = peak response of pseudoephedrine from the *Standard solution*

$C_s$  = concentration of USP Pseudoephedrine Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amounts ( $Q_i$ ) of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) dissolved at each time point ( $i$ ) shown in *Table 3*:

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_s)] + (C_i \times V_s)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times [V - (2 \times V_s)]] + [(C_2 + C_i) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times [V - (3 \times V_s)]] + [(C_3 + C_2 + C_i) \times V_s]\} \times (1/L) \times 100$$

$$\text{Result}_5 = \{[C_5 \times [V - (4 \times V_s)]] + [(C_4 + C_3 + C_2 + C_i) \times V_s]\} \times (1/L) \times 100$$

$C_i$  = concentration of pseudoephedrine hydrochloride in the portion of sample withdrawn at time point ( $i$ ) (mg/mL)

$V$  = volume of the *Medium* (500 mL)

$V_s$  = volume of the *Sample solution* withdrawn from the *Medium* (mL)

$L$  = label claim for pseudoephedrine hydrochloride (mg/Tablet)

#### Tolerances

**Cetirizine hydrochloride:** NLT 75% (Q) of the labeled amount of cetirizine hydrochloride ( $C_{21}H_{25}ClN_2O_3 \cdot 2HCl$ ) is dissolved.

**Pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ):** See *Table 3*.



Table 3

Time Point (h)	Time (h)	Amount Dissolved
1	0.5	—
2	1	30%–50%
3	2	50%–70%
4	4	70%–90%
5	8	NLT 80%

The percentages of the labeled amount of pseudoephedrine hydrochloride dissolved at the times specified conform to *Acceptance Table 2* in (711).

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • CETIRIZINE HYDROCHLORIDE RELATED COMPOUNDS

**Buffer, Diluent, Solution A, and Solution B:** Proceed as directed in the *Assay for Cetirizine hydrochloride*.  
**Mobile phase:** See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	100	0
27	100	0
45	60	40
65	60	40
65.1	100	0
75	100	0

**Standard stock solution:** 0.5 mg/mL of USP Cetirizine Hydrochloride RS in *Diluent*. [NOTE—Sonicate to dissolve.]

**Standard solution:** 1 µg/mL of USP Cetirizine Hydrochloride RS in *Diluent* from the *Standard stock solution*

**Sample stock solution:** 0.5 mg/mL of cetirizine hydrochloride (from NMT 10 finely powdered Tablets) prepared as follows. Dissolve the Tablets first in methanol, using 70% of the final flask volume. Sonicate for 15 min, and then shake for 15 min. Allow the solution to cool to room temperature, and dilute with methanol to volume. Centrifuge a portion for 10 min.

**Sample solution:** 0.2 mg/mL of cetirizine hydrochloride in *Buffer* from the *Sample stock solution*. Pass a portion through a suitable membrane filter of 0.45-µm pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 15-cm; 3.5-µm packing L1

**Temperatures**

**Column:** 30°

**Autosampler:** 5°

**Flow rate:** 1 mL/min

**Injection volume:** 25 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 1300 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 5.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of the individual impurity from the *Sample solution*

$r_S$  = peak response of cetirizine from the *Standard solution*

$C_S$  = concentration of USP Cetirizine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cetirizine hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 5*)

**Acceptance criteria:** See *Table 5*.

[NOTE—Disregard any peak less than 0.05% of the main peak.]

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cetirizineethanol <sup>a</sup>	0.54	1.4	0.3
Chlorobenzhydryl piperazine (CBHP) <sup>b</sup>	0.57	1.5	0.3
Cetirizine	1.0	—	—
Cetirizine acetic acid <sup>c</sup>	1.30	1.1	0.3
Cetirizine N-oxide <sup>d</sup>	1.47	1.2	0.3
Any unspecified degradation product	—	1.0	0.2
Total impurities	—	—	0.8

<sup>a</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethanol.

<sup>b</sup> 1-[4-[(4-Chlorophenyl)phenylmethyl]piperazine.

<sup>c</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]acetic acid.

<sup>d</sup> 2-[4-[(4-Chlorophenyl)phenylmethyl]-1-oxide-1-piperazinyl]ethoxy]acetic acid.

##### • PSEUDOEPHEDRINE HYDROCHLORIDE RELATED COMPOUNDS

**Buffer:** 11.2 g/L of sodium perchlorate monohydrate in water. Adjust with hydrochloric acid to a pH of 2.7.

**Solution A:** Methanol and *Buffer* (3:17)

**Solution B:** Methanol and *Buffer* (1:1)

**Diluent:** *Solution A*

**Mobile phase:** See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
35	28	72

**Standard stock solution:** 0.48 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent*

**Standard solution:** 4.8 µg/mL of USP Pseudoephedrine Hydrochloride RS in *Diluent* from the *Standard stock solution*

**System suitability stock solution:** 49 µg/mL of ephedrine in *Diluent* from USP Ephedrine Sulfate RS

**System suitability solution:** 1.96 µg/mL of ephedrine and 0.46 mg/mL of USP Pseudoephedrine Hydrochloride RS in *Standard stock solution* from the *System suitability stock solution* and the *Standard stock solution*, respectively

**Sample stock solution:** 2.4 mg/mL of pseudoephedrine hydrochloride (from NMT 25 finely powdered Tablets) prepared as follows. Dissolve the Tablets first in methanol, using 75% of the final flask volume. Sonicate for NLT 15 min, and then shake for 15 min. Allow the solution to cool to room temperature, and dilute with methanol to volume. Centrifuge a portion for 10 min.

**Sample solution:** 0.48 mg/mL of pseudoephedrine hydrochloride in *Diluent* from the *Sample stock solution*. Pass a portion through a suitable membrane filter of 0.45-µm pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 212 nm  
 Column: 4.6-mm × 25-cm; 4-μm packing L11  
 Column temperature: 40°  
 Flow rate: 1 mL/min  
 Injection volume: 30 μL  
 System suitability  
 Samples: Standard solution and System suitability solution  
 Suitability requirements  
 Resolution: NLT 1.3 between ephedrine and pseudoephedrine, System suitability solution  
 Tailing factor: NMT 1.5, Standard solution  
 Relative standard deviation: NMT 3.0%, Standard solution  
 Analysis  
 Samples: Standard solution and Sample solution  
 Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of the individual impurity from the Sample solution  
 $r_s$  = peak response of pseudoephedrine from the Standard solution  
 $C_s$  = concentration of USP Pseudoephedrine Hydrochloride RS in the Standard solution (mg/mL)  
 $C_u$  = nominal concentration of pseudoephedrine hydrochloride in the Sample solution (mg/mL)  
 $F$  = relative response factor (see Table 7)  
 Acceptance criteria: See Table 7.  
 [NOTE—Disregard any peak less than 0.05% of the main peak.]

Table 7

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ephedrine <sup>a,b</sup>	0.95	—	—
Pseudoephedrine	1.0	—	—
Methcathinone <sup>c</sup>	1.1	1.1	0.2
Any unspecified degradation product	—	1.0	0.2
Total pseudoephedrine related impurities	—	—	0.5

<sup>a</sup> [R-(R\*,S\*)]-α-[1-(Methylamino)ethyl]-benzenemethanol.

<sup>b</sup> For system suitability and identification purposes only.

<sup>c</sup> 2-Methylamino-1-phenylpropan-1-one.

Sum of cetirizine and pseudoephedrine related impurities: NMT 1.0%

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- LABELING:** When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.

- USP REFERENCE STANDARDS (11)**  
 USP Cetirizine Hydrochloride RS  
 USP Ephedrine Sulfate RS  
 USP Pseudoephedrine Hydrochloride RS

## Cetylpyridinium Chloride



$C_{21}H_{38}ClN \cdot H_2O$  358.00

$C_{21}H_{38}ClN$  339.99

Pyridinium, 1-hexadecyl-, chloride, monohydrate;  
 1-Hexadecylpyridinium chloride monohydrate [6004-24-6].  
 Anhydrous [123-03-5].

#### DEFINITION

Cetylpyridinium Chloride contains NLT 98.0% and NMT 102.0% of cetylpyridinium chloride ( $C_{21}H_{38}ClN$ ), calculated on the anhydrous basis.

#### IDENTIFICATION

- A. INFRARED ABSORPTION (197K)**
- B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.
- C. IDENTIFICATION TESTS—GENERAL, Chloride (191)**  
 Sample solution: 2 mg/mL in water  
 Acceptance criteria: A 10-mL portion of the Sample solution meets the requirements, except that when silver nitrate TS is added, turbidity is produced rather than a curdy white precipitate.

#### ASSAY

##### PROCEDURE

Use 0.1% trifluoroacetic acid-rinsed glassware and silanized vials for all solutions containing cetylpyridinium chloride, as cetylpyridinium may react with the surface.

**Solution A:** Trifluoroacetic acid and water (1:999)

**Solution B:** Acetonitrile and trifluoroacetic acid (999:1)

**Mobile phase:** Solution A and Solution B (62.5: 37.5)

**Standard solution:** 0.25 mg/mL of USP Cetylpyridinium Chloride RS in Solution A

**Sample solution:** 0.25 mg/mL of Cetylpyridinium Chloride in Solution A

##### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 258 nm

Column: 2.1-mm × 10-cm; 5-μm packing L78

Column temperature: 40°

Flow rate: 0.6 mL/min

Injection volume: 2 μL

##### System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

##### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of cetylpyridinium chloride ( $C_{21}H_{38}ClN$ ) in the portion of Cetylpyridinium Chloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the Sample solution  
 $r_s$  = peak response from the Standard solution  
 $C_s$  = concentration of USP Cetylpyridinium Chloride RS in the Standard solution (mg/mL)



$C_U$  = concentration of Cetylpyridinium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2% on the anhydrous basis

Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm • (Official 1-Jan-2018)

#### ORGANIC IMPURITIES

Use 0.1% trifluoroacetic acid-rinsed glassware and silanized vials for all solutions containing cetylpyridinium chloride, as cetylpyridinium may react with the surface. **Solution A**, **Solution B**, **Mobile phase**, and **Chromatographic system**: Proceed as directed in the *Assay*.

**Standard solution**: 2.5 µg/mL of USP Cetylpyridinium Chloride RS in *Solution A*

**Sample solution**: 2.5 mg/mL of Cetylpyridinium Chloride in *Solution A*

#### System suitability

**Sample**: *Standard solution*

#### Suitability requirements

Relative standard deviation: NMT 2.0%

#### Analysis

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of each unspecified impurity in the portion of Cetylpyridinium Chloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each unspecified impurity from the *Sample solution*

$r_S$  = peak response of cetylpyridinium from the *Standard solution*

$C_S$  = concentration of USP Cetylpyridinium Chloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cetylpyridinium Chloride in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peaks less than 0.04%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cetylpyridinium chloride	1.0	—
Any individual unspecified impurity	—	0.1
Total impurities	—	1.0

#### SPECIFIC TESTS

##### ACIDITY

**Analysis**: Dissolve 500 mg of sample in 50 mL of water, add phenolphthalein TS, and titrate with 0.020 N sodium hydroxide.

Acceptance criteria: NMT 2.5 mL is required for neutralization.

- **WATER DETERMINATION**, *Method I* (921): 4.5%–5.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in well-closed containers.

- **USP REFERENCE STANDARDS** (11)  
USP Cetylpyridinium Chloride RS

## Cetylpyridinium Chloride Lozenges

#### DEFINITION

Cetylpyridinium Chloride Lozenges contain NLT 90.0% and NMT 125.0% of the labeled amount of cetylpyridinium chloride ( $C_{21}H_{38}ClN \cdot H_2O$ ) in a suitable molded base.

#### IDENTIFICATION

##### A.

**Eluting solvent**: Alcohol and 1.2 N hydrochloric acid (7:3)

**Chromatographic column**: Pack a pledget of fine glass wool in the base of a 10-mm × 200-mm chromatographic tube. Add styrene-divinylbenzene cation-exchange resin (strong acid form) to form a uniform column 12 cm in height, and top the column with a pledget of fine glass wool.

**Standard solution**: 5 µg/mL of USP Cetylpyridinium Chloride RS in *Eluting solvent*

**Sample solution**: Dissolve nominally 500 µg of cetylpyridinium chloride from NLT 20 finely powdered Lozenges in 50 mL of water. Immediately transfer this solution to the *Chromatographic column*, and discard the eluate. Wash the column, successively, with 200 mL of water, 100 mL of alcohol, 100 mL of water, and 100 mL of 3 N hydrochloric acid. Discard the washings. Elute the column with 80 mL of *Eluting solvent*. Collect the eluate in a 100-mL volumetric flask, and dilute with the *Eluting solvent* to volume.

#### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode**: UV

**Wavelength range**: 225–300 nm

#### Analysis

**Samples**: *Standard solution* and *Sample solution*

**Acceptance criteria**: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of the *Standard solution*.

#### ASSAY

##### PROCEDURE

**0.004 M sodium lauryl sulfate**: Dissolve 1.15 g of sodium lauryl sulfate in 500 mL of water. Add 2 mL of sulfuric acid, and dilute with water to 1000 mL.

**Standardization of 0.004 M sodium lauryl sulfate**:

Determine the molarity of the solution as follows. To a glass-stoppered 100-mL cylinder transfer 10.0 mL of 0.004 M cetylpyridinium chloride (1.432 mg/mL of USP Cetylpyridinium Chloride RS). Add 5 mL of 2 N sulfuric acid, 20 mL of chloroform, and 1 mL of methyl yellow TS. Titrate with the sodium lauryl sulfate solution with frequent vigorous shaking until the chloroform layer acquires the first permanent orange-pink color.

Calculate the molarity, and restandardize before each use. [NOTE—Sulfuric acid is included in this solution to inhibit precipitate formation. If a precipitate forms under storage, discard the solution, and prepare and standardize a fresh solution of 0.004 M sodium lauryl sulfate.]

**Sample solution**: Nominally 0.1 mg/mL of cetylpyridinium chloride prepared as follows. Dissolve an accurately determined number of Lozenges (about 100) in about 400 mL of water in a 500-mL volumetric flask, and dilute with water to volume. Transfer a measured aliquot of this solution, equivalent to about 10 mg of cetylpyridinium chloride, to a glass-stoppered, 100-mL cylinder. Add 5 mL of 2 N sulfuric acid, 20 mL of chloroform, and 1 mL of methyl yellow TS. Insert the



stopper, and shake until the chloroform layer develops a bright yellow color.

**Analysis:** Titrate with 0.004 M sodium lauryl sulfate, shaking thoroughly after each addition, until the chloroform layer develops the first permanent orange-pink color. Each mL of 0.004 M sodium lauryl sulfate is equivalent to 1.432 mg of cetylpyridinium chloride ( $C_{21}H_{38}ClN \cdot H_2O$ ).

Acceptance criteria: 90.0%–125.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
USP Cetylpyridinium Chloride RS

### Cetylpyridinium Chloride Topical Solution

#### DEFINITION

Cetylpyridinium Chloride Topical Solution contains NLT 95.0% and NMT 105.0% of the labeled amount of cetylpyridinium chloride ( $C_{21}H_{38}ClN \cdot H_2O$ ).

#### IDENTIFICATION

- **A.**  
Standard solution: 40 µg/mL of USP Cetylpyridinium Chloride RS in water  
Sample solution: 40 µg/mL of cetylpyridinium chloride from Topical Solution diluted with water  
**Analysis**  
Samples: Standard solution and Sample solution  
Acceptance criteria: The UV absorption spectrum of the Sample solution exhibits maxima and minima at the same wavelengths as that of the Standard solution.
- **B. IDENTIFICATION TESTS—GENERAL, Chloride (191)**  
Sample solution: Evaporate on a steam bath a volume of Topical Solution equivalent to 500 mg of cetylpyridinium chloride from Topical Solution to one-half of its original volume.  
Acceptance criteria: The Sample solution meets the requirements, except that when silver nitrate TS is added, turbidity is produced rather than a curdy white precipitate.

#### ASSAY

##### PROCEDURE

**Sample solution:** Add a volume of Topical Solution nominally equivalent to 150 mg of cetylpyridinium chloride to a glass-stoppered, 500-mL graduated cylinder. Add 10 mL of chloroform, 0.4 mL of bromophenol blue solution (1 in 2000), and 5 mL of a freshly prepared solution of sodium bicarbonate (4.2 in 1000).  
**Analysis:** Titrate the Sample solution with 0.02 M sodium tetraphenylboron VS until the blue color disappears from the chloroform layer. Add the last portions of the sodium tetraphenylboron solution dropwise, agitating vigorously after each addition. Each mL of 0.02 M sodium tetraphenylboron is equivalent to 7.160 mg of cetylpyridinium chloride ( $C_{21}H_{38}ClN \cdot H_2O$ ).

Acceptance criteria: 95.0%–105.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
USP Cetylpyridinium Chloride RS

### Activated Charcoal

#### DEFINITION

Activated Charcoal is the residue from the destructive distillation of various organic materials, treated to increase its adsorptive power.

#### IMPURITIES

- **RESIDUE ON IGNITION (281)**  
Sample: 0.50 g  
Acceptance criteria: NMT 4.0%
- **ACID-SOLUBLE SUBSTANCES**  
Sample: 1.0 g  
Analysis: Boil the Sample with a mixture of 20 mL of water and 5 mL of hydrochloric acid for 5 min. Filter into a tared porcelain crucible, and wash the residue with 10 mL of hot water, adding the washing to the filtrate. To the combined filtrate and washing add 1 mL of sulfuric acid. Evaporate to dryness, and ignite to constant weight.  
Acceptance criteria: The residue weighs NMT 35 mg (NMT 3.5%).
- **CHLORIDE AND SULFATE, Chloride (221)**  
Sample solution: A 10-mL portion of the filtrate obtained in the test for Reaction  
Acceptance criteria: The Sample solution shows no more chloride than is contained in 1.5 mL of 0.020 N hydrochloric acid (NMT 0.2%).
- **CHLORIDE AND SULFATE, Sulfate (221)**  
Sample solution: A 10-mL portion of the filtrate obtained in the test for Reaction  
Acceptance criteria: The Sample solution shows no more sulfate than is contained in 1.0 mL of 0.020 N sulfuric acid (NMT 0.2%).
- **SULFIDE**  
Sample: 0.50 g  
Analysis: Place the Sample in a small conical flask. Add 20 mL of water and 5 mL of hydrochloric acid, and boil gently.  
Acceptance criteria: The escaping vapors do not darken paper moistened with lead acetate TS.
- **CYANOGEN COMPOUNDS**  
Sample: 5 g  
Analysis: Place the Sample, 50 mL of water, and 2 g of tartaric acid in a distilling flask connected to a condenser provided with a tightly fitting adapter, the end of which dips below the surface of a mixture of 2 mL of 1 N sodium hydroxide and 10 mL of water, contained in a small flask surrounded by ice. Heat the mixture in the distilling flask to boiling, and distill about 25 mL. Dilute the distillate with water to 50 mL, and mix. To 25 mL of the diluted distillate add 12 drops of ferrous sulfate TS, heat the mixture almost to boiling, cool, and add 1 mL of hydrochloric acid.  
Acceptance criteria: No blue color is produced.

#### Delete the following:

- **HEAVY METALS (231)**  
Sample: 1.0 g  
Test preparation: Boil the Sample with a mixture of 20 mL of 3 N hydrochloric acid and 5 mL of bromine TS for 5 min. Filter, and wash the charcoal and the filter with 50 mL of boiling water. Evaporate the filtrate and



washing to dryness, and to the residue add 1 mL of 1 N hydrochloric acid, 20 mL of water, and 5 mL of sulfuric acid. Boil the solution until all of the sulfur dioxide is expelled. Filter if necessary, and dilute with water to 50 mL. To 20 mL of the solution add water to make 25 mL.

Acceptance criteria: NMT 50 ppm. (Official 1-Jan-2018)

#### • UNCARBONIZED CONSTITUENTS

Sample: 0.25 g

Analysis: Boil the Sample with 10 mL of 1 N sodium hydroxide for 5 s, and filter.

Acceptance criteria: The filtrate is colorless.

#### SPECIFIC TESTS

##### • ADSORPTIVE POWER

###### Alkaloids

Sample: 1 g, previously dried at 120° for 4 h

Analysis: Shake the Sample with a solution of 100 mg of strychnine sulfate in 50 mL of water for 5 min, and filter through a dry filter, rejecting the first 10 mL of the filtrate. To a 10-mL portion of the subsequent filtrate add 1 drop of hydrochloric acid and 5 drops of mercuric-potassium iodide TS.

Acceptance criteria: No turbidity is produced.

###### Dyes

Sample: 250 mg

Analysis: Pipet 50 mL of methylene blue solution (1 in 1000) into each of two glass-stoppered, 100-mL flasks. Add the Sample to one of the flasks, insert the stopper in the flask, and shake for 5 min. Filter the contents of each flask through a dry filter, rejecting the first 20 mL of each filtrate. Pipet 25-mL portions of the remaining filtrates into two 250-mL volumetric flasks. Add to each flask 50 mL of sodium acetate solution (1 in 10), mix, and add from a buret 35.0 mL of 0.1 N iodine VS, swirling the mixture during the addition. Insert the stoppers in the flasks, and allow them to stand for 50 min, shaking them vigorously at 10-min intervals. Dilute each mixture with water to volume, mix, allow to stand for 10 min, and filter through dry filters, rejecting the first 30 mL of each filtrate. Titrate the excess iodine in a 100-mL aliquot of each subsequent filtrate with 0.1 N sodium thiosulfate VS, adding 3 mL of starch TS as the endpoint is approached. Calculate the mL of 0.1 N iodine consumed in each titration.

Acceptance criteria: The difference between the two volumes is NLT 0.7 mL.

##### • MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):

It meets the requirements of the tests for absence of *Salmonella* species and *Escherichia coli*.

##### • REACTION

Sample: 3.0 g

Analysis: Boil the Sample with 60 mL of water for 5 min. Allow to cool, restore the original volume by the addition of water, and filter.

Acceptance criteria: The filtrate is colorless and is neutral to litmus.

##### • LOSS ON DRYING (731):

Dry a sample at 120° for 4 h: it loses NMT 15.0% of its weight.

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE:

Preserve in well-closed containers.

1,1-Ethanediol, 2,2,2-trichloro-.  
Chloral hydrate [302-17-0].

» Chloral Hydrate contains not less than 99.5 percent and not more than 102.5 percent of  $C_2H_3Cl_3O_2$ .

**Packaging and storage**—Preserve in tight containers.

**Identification**—Transfer to a 125-mL conical flask a portion of a solution in water equivalent to about 1 mg of chloral hydrate, and add water to bring the volume to about 10 mL. Add 10 mL of 1-ethylquinaldinium iodide solution (15 in 1000), which has been filtered through a 0.45- $\mu$ m filter. Add 60 mL of isopropyl alcohol, 5 mL of an aqueous 0.1 M monoethanolamine solution, and 15 mL of water. Mix, and heat in a water bath at 60° for 15 minutes: a blue color develops.

**Acidity**—A 1 in 20 solution in alcohol does not at once redden moistened blue litmus paper.

**Residue on ignition** (281): not more than 0.1%.

**Chloride** (221)—To a 1 in 10 solution in alcohol add a few drops of silver nitrate TS: any opalescence produced does not exceed that of a control containing 0.10 mL of 0.020 N hydrochloric acid (0.007%).

**Readily carbonizable substances** (271)—Shake 500 mg, at intervals of 5 minutes during 1 hour, with 5 mL of sulfuric acid in a glass-stoppered cylinder that previously has been rinsed with sulfuric acid, and transfer the mixture to a comparison vessel: the mixture has no more color than Matching Fluid P.

**Assay**—Dissolve about 4 g of Chloral Hydrate, accurately weighed, in 10 mL of water, add 30.0 mL of 1 N sodium hydroxide VS, and allow the mixture to stand for 2 minutes. Add a few drops of phenolphthalein TS, and titrate the residual alkali at once with 1 N sulfuric acid VS. Each mL of 1 N sodium hydroxide corresponds to 165.4 mg of  $C_2H_3Cl_3O_2$ .

## Chloral Hydrate Capsules

» Chloral Hydrate Capsules contain not less than 95.0 percent and not more than 110.0 percent of the labeled amount of  $C_2H_3Cl_3O_2$ .

**Packaging and storage**—Preserve in tight containers, preferably at controlled room temperature.

**Identification**—The contents of the Capsules respond to the Identification test under Chloral Hydrate.

##### Dissolution (711)—

Medium: water; 500 mL.

Apparatus 2: 50 rpm.

Time: 15 minutes.

**Procedure**—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each capsule shell to rupture.

**Tolerances**—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than 30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—Place a counted number of Capsules, equivalent to about 2.5 g of chloral hydrate, in a glass-stoppered, 250-mL flask, add 25 mL of water, insert the stopper in the flask,

## Chloral Hydrate



$C_2H_3Cl_3O_2$  165.40



and heat on a steam bath with frequent swirling until the Capsules are dissolved. Cool to room temperature, add 25 mL of neutralized alcohol and 20.0 mL of 1 N sodium hydroxide VS, mix, and allow the mixture to stand for 4 minutes. Add phenolphthalein TS, and titrate the excess alkali with 1 N sulfuric acid VS. Each mL of 1 N sodium hydroxide is equivalent to 165.4 mg of  $C_2H_3Cl_3O_2$ .

## Chloral Hydrate Oral Solution

» Chloral Hydrate Oral Solution contains not less than 95.0 percent and not more than 110.0 percent of the labeled amount of chloral hydrate ( $C_2H_3Cl_3O_2$ ).

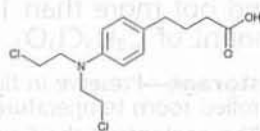
**Packaging and storage**—Preserve in tight, light-resistant containers.

**Identification**—It meets the requirements for the *Identification* test under *Chloral Hydrate*.

**Assay**—Transfer 25.0 mL of Oral Solution to a 250-mL conical flask with the aid of several portions of water. Add 30.0 mL of 1 N sodium hydroxide VS, and mix. After the mixture has stood for 2 minutes, add 5 drops of phenolphthalein TS, and immediately titrate the excess sodium hydroxide with 1 N sulfuric acid VS. Designate the volume of 1 N sodium hydroxide VS consumed as *A*. Transfer 5.0 mL of Oral Solution to a second 250-mL conical flask with the aid of several portions of water. Add 10 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide VS. Designate the volume of 0.1 N sodium hydroxide VS consumed as *B*. Calculate the weight, in mg, of chloral hydrate ( $C_2H_3Cl_3O_2$ ) in the amount of Oral Solution taken by the first titration by the formula:

$$165.4(A - 0.5B).$$

## Chlorambucil



$C_{14}H_{19}Cl_2NO_2$  304.21  
Benzenebutanoic acid, 4-[bis(2-chloroethyl)amino]-;  
4-[*p*-(Bis(2-chloroethyl)amino)phenyl]butyric acid  
[305-03-3].

### DEFINITION

Chlorambucil contains NLT 98.0% and NMT 101.0% of chlorambucil ( $C_{14}H_{19}Cl_2NO_2$ ), calculated on the anhydrous basis.

[**CAUTION**—Great care should be taken to prevent inhaling particles of Chlorambucil and exposing the skin to it.]

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197S)

**Sample solution:** 8 mg/mL in carbon disulfide

**Cell:** 1 mm

**Acceptance criteria:** Meets the requirements

#### B.

**Sample solution:** Dissolve 50 mg of Chlorambucil in 5 mL of acetone, and dilute with water to 10 mL.

**Analysis:** To the *Sample solution* add 1 drop of 2 N sulfuric acid, then add 4 drops of silver nitrate TS.

**Acceptance criteria:** No opalescence is observed immediately (absence of chloride ion). Warm the solution on a steam bath: opalescence develops (presence of ionizable chlorine).

### ASSAY

#### PROCEDURE

**Sample:** 200 mg of Chlorambucil

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N sodium hydroxide VS

**Endpoint detection:** Visual

**Analysis:** Dissolve the *Sample* in 10 mL of acetone, add 10 mL of water, and titrate with *Titrant*, using phenolphthalein TS as the indicator. Each mL of 0.1 N sodium hydroxide is equivalent to 30.42 mg of chlorambucil ( $C_{14}H_{19}Cl_2NO_2$ ).

**Acceptance criteria:** 98.0%–101.0% on the anhydrous basis

### SPECIFIC TESTS

• **MELTING RANGE OR TEMPERATURE (741):** 65°–69°

• **WATER DETERMINATION, Method I (921):** NMT 0.5%

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**  
USP Chlorambucil RS

## Chlorambucil Tablets

### DEFINITION

Chlorambucil Tablets contain NLT 85.0% and NMT 110.0% of the labeled amount of chlorambucil ( $C_{14}H_{19}Cl_2NO_2$ ).

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197S)

**Sample solution:** Shake a quantity of finely powdered Tablets, equivalent to 16 mg of chlorambucil, with 20 mL of carbon disulfide. Filter, evaporate to dryness, and dissolve the residue in 2 mL of carbon disulfide.

**Cell:** 1 mm

**Acceptance criteria:** Meet the requirements

### ASSAY

#### PROCEDURE

**Mobile phase:** Mix 500 mL of alcohol with 1.0 mL of glacial acetic acid. Dilute with water to 1 L. Degas the solution at a pressure of approximately 250 mm of mercury for 2 min. [NOTE—The alcohol concentration may be varied to meet system suitability requirements and to provide a suitable elution time for chlorambucil.]

**Internal standard solution:** 0.4 mg/mL of USP Propylparaben RS in alcohol

**Standard stock solution:** 1 mg/mL of USP Chlorambucil RS in alcohol

**Standard solution:** 0.02 mg/mL of USP Chlorambucil RS in alcohol prepared as follows. Transfer 2.0 mL of the *Standard stock solution* into a 100-mL volumetric flask containing 50 mL of alcohol and, while gently swirling, add 5.0 mL of 0.1 N hydrochloric acid and 2.0 mL of *Internal standard solution*. Dilute with alcohol to volume.

**Sample solution:** Nominally 0.02 mg/mL of chlorambucil in alcohol prepared as follows. Transfer finely powdered Tablets (NLT 20), equivalent to 2 mg of chlorambucil, into a 100-mL volumetric flask containing 50 mL of alcohol and, while gently swirling, add 5.0 mL of 0.1 N hydrochloric acid and 2.0 mL of *Internal standard solution*. Sonicate for 5 min, and dilute with alco-



hol to volume. Filter through a medium pore size, sintered-glass filtering funnel, maintaining reduced pressure for the minimum necessary time to avoid solvent loss from evaporation.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 25- or 30-cm × 2-mm; 5- to 10-μm packing L1

Flow rate: Capable of giving the required *Resolution* in *Suitability requirements* and a suitable elution time

Injection volume: 10–12 μL

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Resolution: NLT 2.0 between the propylparaben and chlorambucil peaks

Relative standard deviation: NMT 2.0% for 6–8 injections, for the peak response ratio of chlorambucil to propylparaben

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of chlorambucil

(C<sub>14</sub>H<sub>19</sub>Cl<sub>2</sub>NO<sub>2</sub>) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of chlorambucil to propylparaben from the *Sample solution*

$R_S$  = peak response ratio of chlorambucil to propylparaben from the *Standard solution*

$C_S$  = concentration of USP Chlorambucil RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of chlorambucil in the *Sample solution* (mg/mL)

Acceptance criteria: 85.0%–110.0%

### PERFORMANCE TESTS

#### • DISINTEGRATION (701)

**Analysis:** Place 1 Tablet in each of the six tubes of the basket, and if the Tablet has a soluble external coating, immerse the basket in water at room temperature for 5 min. Operate the apparatus, using simulated gastric fluid TS maintained at 37 ± 2° as the immersion fluid. After 30 min of operation in simulated gastric fluid TS, lift the basket from the fluid, and observe the Tablets. If the Tablets have not disintegrated completely, substitute simulated intestinal fluid TS maintained at 37 ± 2° as the immersion fluid, and continue the test for a total period of time equal to 45 min, including previous exposure to water and simulated gastric fluid TS. Lift the basket from the fluid, and observe the Tablets.

**Acceptance criteria:** All of the Tablets have disintegrated completely. If 1 or 2 Tablets fail to disintegrate completely, repeat the test on 12 additional Tablets: NLT 16 of the total of 18 Tablets tested disintegrate completely.

#### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE:

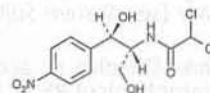
Preserve coated Tablets in well-closed containers. Preserve uncoated Tablets in well-closed, light-resistant containers.

#### • USP REFERENCE STANDARDS (11)

USP Chlorambucil RS

USP Propylparaben RS

## Chloramphenicol



C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub> 323.13

Acetamide, 2,2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]-, [R-(R\*,R\*)]-.

D-threo-(-)-2,2-Dichloro-N-[β-hydroxy-α-(hydroxymethyl)-p-nitrophenethyl]acetamide [56-75-7].

» Chloramphenicol contains not less than 97.0 percent and not more than 103.0 percent of C<sub>11</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>5</sub>.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing injectable or other sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable or other sterile dosage forms.

#### USP Reference standards (11)—

USP Chloramphenicol RS

USP Endotoxin RS

#### Identification—

A: *Infrared Absorption* (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Melting range** (741): between 149° and 153°.

**Specific rotation** (781S): between +17.0° and +20.0°.

*Test solution:* 50 mg, undried, per mL, in dehydrated alcohol.

**Crystallinity** (695): meets the requirements.

**Bacterial Endotoxins Test** (85)—Where Chloramphenicol is intended for use in preparing injectable dosage forms, it contains not more than 0.2 USP Endotoxin Unit per mg of chloramphenicol.

**Sterility Tests** (71)—Where the label states that Chloramphenicol is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, except to use 1 g of solid specimen.

**pH** (791): between 4.5 and 7.5, in an aqueous suspension containing 25 mg per mL.

**Chromatographic purity**—Dissolve an accurately weighed quantity of Chloramphenicol in methanol to obtain a test solution containing 10 mg per mL. Prepare a solution of USP Chloramphenicol RS in methanol containing 10 mg per mL (*Standard solution A*). Dilute portions of *Standard solution A* quantitatively with methanol to obtain *Standard solution B* containing 100 μg per mL and *Standard solution C* containing 50 μg per mL. Apply separate 20-μL portions of the test solution and *Standard solutions B* and *C* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)), coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and glacial acetic acid (79:14:7) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and examine under short-wavelength UV light: any spot other than the principal spot obtained from the test solution does not ex-



ceed in size or intensity the principal spot obtained from *Standard solution B* (1%), and the sum of the impurities represented by all of the spots other than the principal spot, based on a comparison of the intensities of such spots with the intensities of the principal spots obtained from *Standard solutions B* and *C*, does not exceed 2%.

#### Assay—

**Mobile phase**—Prepare a suitable filtered mixture of water, methanol, and glacial acetic acid (55:45:0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 80 µg per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

**Assay preparation**—Transfer about 200 mg of Chloramphenicol, accurately weighed, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 4.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 4.6-mm × 10-cm column that contains 5-µm packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 1800 theoretical plates, the tailing factor is not more than 2.0, and the relative standard deviation for replicate injections is not more than 1.0%.

**Procedure**—[NOTE—Use peak heights where peak responses are indicated.] Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in the portion of Chloramphenicol taken by the formula:

$$2.5C(r_U / r_S)$$

in which *C* is the concentration, in µg per mL, of USP Chloramphenicol RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chloramphenicol Capsules

» Chloramphenicol Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ .

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

#### Dissolution (711)—

**Medium**: 0.01 N hydrochloric acid; 900 mL.

**Apparatus 1**: 100 rpm.

**Time**: 30 minutes.

**Procedure**—Determine the amount of  $C_{11}H_{12}Cl_2N_2O_5$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 278 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Chloramphenicol RS in the same *Medium*.

**Tolerances**—Not less than 85% (*Q*) of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay* under *Chloramphenicol*.

**Standard preparation**—Transfer about 25 mg of USP Chloramphenicol RS, accurately weighed, to a 200-mL volumetric flask, add 10 mL of water, and heat on a steam bath until completely dissolved. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

**Assay preparation**—Transfer an accurately counted number of Chloramphenicol Capsules, equivalent to about 2500 mg of chloramphenicol, to a 1000-mL volumetric flask, add 100 mL of water, and heat on a steam bath until the Capsules have disintegrated. Add 300 mL of water, and heat on a steam bath for 20 minutes, with occasional mixing. Cool to room temperature, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in each Capsule taken by the formula:

$$20(C / N)(r_U / r_S)$$

in which *N* is the number of Capsules taken, and the other terms are as defined therein.

## Chloramphenicol Cream

» Chloramphenicol Cream contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ .

**Packaging and storage**—Preserve in collapsible tubes or in tight containers.

#### USP Reference standards (11)—

USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Minimum fill** (755): meets the requirements.

#### Assay—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay* under *Chloramphenicol*.

**Standard preparation**—Transfer about 40 mg of USP Chloramphenicol RS, accurately weighed, to a 100-mL volumetric flask, dissolve in methanol, dilute with methanol to



volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

**Assay preparation**—Transfer an accurately weighed quantity of Chloramphenicol Cream, equivalent to about 40 mg of chloramphenicol, to a 100-mL volumetric flask, add about 80 mL of methanol, and sonicate for about 10 minutes. Cool to room temperature, dilute with methanol to volume, and mix. Transfer 10.0 mL of the resulting solution to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in the portion of Cream taken by the formula:

$$0.5C(r_u / r_s)$$

in which the terms are as defined therein.

## Chloramphenicol Injection

» Chloramphenicol Injection is a sterile solution of Chloramphenicol in one or more suitable solvents. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ . It may contain suitable buffers.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

### USP Reference standards (11)—

USP Chloramphenicol RS

USP Endotoxin RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of chloramphenicol.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 1 mL from each container being transferred directly to the membrane filter.

**pH** (791): between 5.0 and 8.0, in a solution diluted with water (1:1).

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

### Assay—

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Prepare as directed in the *Assay* under *Chloramphenicol*.

**Assay preparation**—Transfer an accurately measured volume of Chloramphenicol Injection, equivalent to about 200 mg of chloramphenicol, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 4.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter this solution through a 0.5- $\mu$ m or finer porosity filter.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of

$C_{11}H_{12}Cl_2N_2O_5$  in each mL of the Injection taken by the formula:

$$2.5(C / V)(r_u / r_s)$$

in which *V* is the volume, in mL, of Injection taken, and the other terms are as defined therein.

## Chloramphenicol Ophthalmic Ointment

### DEFINITION

Chloramphenicol Ophthalmic Ointment contains NLT 90.0% and NMT 130.0% of the labeled amount of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** Methanol, glacial acetic acid, and water (450:1:550)

**Standard stock solution:** 0.25 mg/mL of USP Chloramphenicol RS in methanol

**Standard solution:** 0.1 mg/mL of USP Chloramphenicol RS from the *Standard stock solution* in *Mobile phase*. Pass through a suitable filter, and use the clear filtrate.

**Sample stock solution:** Nominally 0.25 mg/mL of chloramphenicol prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 25 mg of chloramphenicol to a suitable conical flask. Add 20 mL of cyclohexane, mix, and sonicate for 2 min. Add 60 mL of methanol. Filter this mixture, collecting the filtrate in a 100-mL volumetric flask. Wash the filter with methanol, collecting the washings in the volumetric flask. Dilute with methanol to volume. Transfer 50.0 mL of the resulting solution to a suitable round-bottom flask, and evaporate to dryness by rotating the flask under vacuum in a water bath at 35°. Dissolve the residue in 50.0 mL of methanol.

**Sample solution:** Nominally 0.1 mg/mL of chloramphenicol from the *Sample stock solution* in *Mobile phase*. Pass through a suitable filter, and use the clear filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 1.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times P \times F \times 100$$

$r_u$  = peak height from the *Sample solution*

$r_s$  = peak height from the *Standard solution*

$C_s$  = concentration of USP Chloramphenicol RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of chloramphenicol in the *Sample solution* (mg/mL)



$P$  = potency of chloramphenicol in USP Chloramphenicol RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001  $\text{mg}/\mu\text{g}$   
 Acceptance criteria: 90.0%–130.0%

**SPECIFIC TESTS**

- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS**: It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** (11)  
USP Chloramphenicol RS

**Chloramphenicol Ophthalmic Solution**

» Chloramphenicol Ophthalmic Solution is a sterile solution of Chloramphenicol. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of  $\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_5$ .

**Packaging and storage**—Preserve in tight containers, and store in a refrigerator until dispensed. The containers or individual cartons are sealed and tamper-proof so that sterility is assured at time of first use.

**Labeling**—The labeling states that there is a 21-day beyond-use period after dispensing.

**USP Reference standards** (11)—  
USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 7.0 and 7.5, except that in the case of Ophthalmic Solution that is unbuffered or is labeled for veterinary use it is between 3.0 and 6.0.

**Assay—**

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay under Chloramphenicol*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100  $\mu\text{g}$  per mL. Filter a portion of this solution through a 0.5- $\mu\text{m}$  or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 50 mg of chloramphenicol, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu\text{m}$  or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure in the Assay under Chloramphenicol*. Calculate the quantity, in mg, of

$\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_5$  in each mL of the Ophthalmic Solution taken by the formula:

$$0.5(C/V)(r_u/r_s)$$

in which  $V$  is the volume, in mL, of Ophthalmic Solution taken, and the other terms are as defined therein.

**Chloramphenicol for Ophthalmic Solution**

» Chloramphenicol for Ophthalmic Solution is a sterile, dry mixture of Chloramphenicol with or without one or more suitable buffers, diluents, and preservatives. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of  $\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_5$ , when constituted as directed.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—If packaged in combination with a container of solvent, label it with a warning that it is not for injection.

**USP Reference standards** (11)—  
USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 7.1 and 7.5, in an aqueous solution containing 5 mg of chloramphenicol per mL.

**Assay—**

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay under Chloramphenicol*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100  $\mu\text{g}$  per mL. Filter a portion of this solution through a 0.5- $\mu\text{m}$  or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Transfer the contents of 1 container of Chloramphenicol for Ophthalmic Solution to a suitable container with the aid of *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a concentration of about 100  $\mu\text{g}$  of chloramphenicol per mL. Filter a portion of this solution through a 0.5- $\mu\text{m}$  or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure in the Assay under Chloramphenicol*. Calculate the quantity, in mg, of  $\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_5$  in the container of Chloramphenicol for Ophthalmic Solution taken by the formula:

$$(L/D)(C/r_u/r_s)$$

in which  $L$  is the labeled quantity, in mg, of chloramphenicol in the container,  $D$  is the concentration, in  $\mu\text{g}$  per mL, of chloramphenicol in the *Assay preparation*, based on the labeled quantity and the extent of dilution, and the other terms are as defined therein.



## Chloramphenicol Oral Solution

» Chloramphenicol Oral Solution is a solution of Chloramphenicol in a suitable solvent. It contains one or more suitable buffers and preservatives. It has a potency of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ .

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only and that it is not to be used in animals raised for food production.

**USP Reference standards** (11)—

USP Chloramphenicol RS

**Identification**—Prepare a *Test solution* containing 20 µg per mL chloramphenicol from Oral Solution diluted with water. The ultraviolet absorption spectrum of the *Test solution* exhibits maxima and minima only at the same wavelength as that of a similar solution of USP Chloramphenicol RS, concomitantly measured.

**pH** (791): between 5.0 and 8.5, when diluted with an equal volume of water.

**Assay**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of Oral Solution diluted quantitatively and stepwise with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Chloramphenicol Otic Solution

» Chloramphenicol Otic Solution is a sterile solution of Chloramphenicol in a suitable solvent. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.0 and 8.0, when diluted with an equal volume of water.

**Water Determination, Method I** (921): not more than 2.0%.

**Assay**—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 100 µg per mL. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Transfer an accurately measured volume of Otic Solution, equivalent to about 50 mg of chlor-

amphenicol, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in each mL of the Otic Solution taken by the formula:

$$0.5(C/V)(r_U/r_S)$$

in which *V* is the volume, in mL, of Otic Solution taken, and the other terms are as defined therein.

## Chloramphenicol Tablets

» Chloramphenicol Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{11}H_{12}Cl_2N_2O_5$ .

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label Tablets to indicate that they are for veterinary use only and are not to be used in animals raised for food production.

**USP Reference standards** (11)—

USP Chloramphenicol RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Disintegration** (701): 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

*Standard preparation*—Transfer about 25 mg of USP Chloramphenicol RS, accurately weighed, to a 200-mL volumetric flask, add 10 mL of water, and heat on a steam bath until completely dissolved. Cool to room temperature, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 500 mg of chloramphenicol, to a 200-mL volumetric flask, add 80 mL of water, and heat on a steam bath for 20 minutes, with occasional mixing. Cool to room temperature, dilute with water to volume, and mix. Transfer 5.0 mL of the resulting solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5-µm or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of  $C_{11}H_{12}Cl_2N_2O_5$  in the portion of Tablets taken by the formula:

$$4C(r_U/r_S)$$

in which the terms are as defined therein.



## Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Suspension

» Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Suspension is a sterile, dry mixture of Chloramphenicol and Hydrocortisone Acetate with or without one or more suitable buffers, diluents, and preservatives. It contains not less than 90.0 percent and not more than 130.0 percent of the labeled amount of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ), and not less than 90.0 percent and not more than 115.0 percent of the labeled amount of hydrocortisone acetate ( $C_{23}H_{32}O_6$ ), when constituted as directed.

**Labeling**—If packaged in combination with a container of solvent, label it with a warning that it is not for injection.

### USP Reference standards (11)—

USP Chloramphenicol RS

USP Hydrocortisone Acetate RS

**Identification**—The retention times of the major peaks in the chromatogram of the *Assay preparation* correspond to that in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*.

**pH** (791): between 7.1 and 7.5, in an aqueous suspension containing 5 mg of chloramphenicol per mL.

### Assay—

*Mobile phase and Chromatographic system*—Proceed as directed in the *Assay* under *Chloramphenicol*.

*Standard preparation*—Transfer about 37.5 mg of USP Chloramphenicol RS and 37.5 mg of USP Hydrocortisone Acetate RS, both accurately weighed, *I* being the ratio of the labeled amount, in mg, of hydrocortisone acetate to the labeled amount, in mg, of chloramphenicol in the Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Solution, to a 100-mL volumetric flask, add 15 mL of water and 75 mL of methanol, sonicate for a few seconds, dilute with methanol to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Standard preparation*.

*Assay preparation*—Transfer the contents of an accurately counted number of containers of Chloramphenicol and Hydrocortisone Acetate for Ophthalmic Solution, equivalent to about 37.5 mg of chloramphenicol, to a 100-mL volumetric flask with the aid of 5 mL of water for each 12.5 mg of chloramphenicol contained therein. Wash each container with methanol, and add the washings to the volumetric flask. Dilute with methanol to volume, and mix. Transfer 5.0 mL of the resulting solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.5- $\mu$ m or finer porosity filter, and use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Chloramphenicol*. Calculate the quantity, in mg, of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) in each container taken by the formula:

$$0.5(C/N)(r_U/r_S)$$

in which *N* is the number of containers taken, and the other terms are as defined therein. Calculate the quantity, in mg,

of hydrocortisone acetate ( $C_{23}H_{32}O_6$ ) in each container taken by the formula:

$$500(C/N)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Hydrocortisone Acetate RS in the *Standard preparation*, *N* is the number of containers taken, and *r<sub>U</sub>* and *r<sub>S</sub>* are the hydrocortisone acetate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chloramphenicol and Polymyxin B Sulfate Ophthalmic Ointment

### DEFINITION

Chloramphenicol and Polymyxin B Sulfate Ophthalmic Ointment contains NLT 90.0% and NMT 120.0% of the labeled amount of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) and NLT 90.0% and NMT 125.0% of the labeled amount of polymyxin B.

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • CHLORAMPHENICOL

**Mobile phase:** Methanol, glacial acetic acid, and water (450:1:550)

**Standard stock solution:** 0.25 mg/mL of USP Chloramphenicol RS in methanol

**Standard solution:** 0.1 mg/mL of USP Chloramphenicol RS from the *Standard stock solution* in *Mobile phase*.

Pass through a suitable filter, and use the clear filtrate.

**Sample stock solution:** Nominally 0.25 mg/mL of chloramphenicol prepared as follows. Transfer a portion of Ophthalmic Ointment containing nominally 25 mg of chloramphenicol to a suitable conical flask. Add 20 mL of cyclohexane, mix, and sonicate for 2 min.

Add 60 mL of methanol. Filter this mixture, collecting the filtrate in a 100-mL volumetric flask. Wash the filter with methanol, collecting the washings in the volumetric flask. Dilute with methanol to volume. Transfer 50.0 mL of the resulting solution to a suitable round-bottom flask, and evaporate to dryness by rotating the flask under vacuum in a water bath at 35°. Dissolve the residue in 50.0 mL of methanol.

**Sample solution:** Nominally 0.1 mg/mL of chloramphenicol from the *Sample stock solution* in *Mobile phase*. Pass through a suitable filter, and use the clear filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 1.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

*r<sub>U</sub>* = peak height from the *Sample solution*



- $r_s$  = peak height from the *Standard solution*  
 $C_s$  = concentration of USP Chloramphenicol RS in the *Standard solution* (mg/mL)  
 $C_u$  = nominal concentration of chloramphenicol in the *Sample solution* (mg/mL)  
 $P$  = potency of chloramphenicol in USP Chloramphenicol RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

Acceptance criteria: 90.0%–120.0%

#### • POLYMYXIN B

(See *Antibiotics—Microbial Assays* (81).)

**Sample solution:** Shake a portion of Ophthalmic Ointment containing nominally 5000 Polymyxin B Units with 50 mL of ether in a separator. Extract with four 20-mL portions of *Buffer B.6*. Combine the aqueous extracts in a 100-mL volumetric flask, and dilute with *Buffer B.6* to volume.

**Analysis:** Proceed as directed in the chapter. Dilute the *Sample solution* with *Buffer B.6* to obtain a *Test Dilution* having a concentration that is nominally equivalent to the median level of the standard.

Acceptance criteria: 90.0%–125.0%

#### SPECIFIC TESTS

- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** (11)  
 USP Chloramphenicol RS  
 USP Polymyxin B Sulfate RS

### Chloramphenicol Palmitate

$\text{C}_{27}\text{H}_{42}\text{Cl}_2\text{N}_2\text{O}_6$  561.54

Hexadecanoic acid, 2-[(2,2-dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl ester, [*R*-(*R*\*,*R*\*)]-.

*D*-threo-(-)-2,2-Dichloro-*N*-[ $\beta$ -hydroxy- $\alpha$ -(hydroxymethyl)-*p*-nitrophenethyl]acetamide  $\alpha$ -palmitate [530-43-8].

» Chloramphenicol Palmitate has a potency equivalent to not less than 555  $\mu\text{g}$  and not more than 595  $\mu\text{g}$  of chloramphenicol ( $\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_5$ ) per mg.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Chloramphenicol Palmitate RS

**Identification**—The retention time of the chloramphenicol palmitate peak in the chromatogram of the *Assay preparation* corresponds to that of the chloramphenicol palmitate peak in the chromatogram of the *Standard preparation* as obtained in the *Assay*.

**Melting range** (741): between 87° and 95°.

**Specific rotation** (781S): between +21° and +25°.

*Test solution:* 50 mg, undried, per mL, in dehydrated alcohol.

**Crystallinity** (695): meets the requirements.

**Loss on drying** (731)—Dry it to constant weight over phosphorus pentoxide in vacuum at a pressure not exceeding 5 mm of mercury: it loses not more than 0.5% of its weight.

**Acidity**—Dissolve 1.0 g by heating at 35° with 5 mL of a 1:1 mixture of 80 percent alcohol and ether, previously neutralized using phenolphthalein TS. Titrate with 0.1 N sodium hydroxide VS, using phenolphthalein TS, until on gentle

shaking a pink color persists for not less than 30 seconds: not more than 0.4 mL is consumed.

**Free chloramphenicol**—Dissolve 1.0 g in 80 mL of xylene with the aid of gentle warming. Cool, and extract with three 15-mL portions of water, combining the aqueous extracts and discarding the xylene. Dilute the combined aqueous extracts with water to 50 mL, extract with 10 mL of toluene, allow to separate, and discard the toluene. Centrifuge a portion of the aqueous solution, and determine the absorbance of the clear solution at the wavelength of maximum absorbance at about 278 nm, using a suitable spectrophotometer, and using as a reagent blank to set the instrument to zero the solution obtained by the same procedure without the specimen: the absorbance is not more than 0.268 (0.045%).

#### Assay—

**Mobile phase**—Prepare a suitable degassed mixture of methanol, water, and glacial acetic acid (172:27:1).

**Standard preparation**—Transfer about 65 mg of USP Chloramphenicol Palmitate RS to a 50-mL volumetric flask, add about 40 mL of methanol and 1 mL of glacial acetic acid, and sonicate for a few minutes. Dilute with methanol to volume, and mix. Transfer 10.0 mL of this solution to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Assay preparation**—Using about 65 mg of Chloramphenicol Palmitate, accurately weighed, prepare as directed under *Standard preparation*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 280-nm detector and a 3.9-mm  $\times$  30-cm column that contains 10- $\mu\text{m}$  packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the column efficiency determined from the analyte peak is not less than 2400 theoretical plates, and the relative standard deviation for replicate injections is not more than 0.5%.

**Procedure**—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu\text{g}$ , of chloramphenicol ( $\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_5$ ) equivalent in each mg of specimen taken by the formula:

$$(W_s / W_u)(P_s)(r_u / r_s)$$

in which  $W_s$  and  $W_u$  are the quantities, in mg, of USP Chloramphenicol Palmitate RS and Chloramphenicol Palmitate taken, respectively;  $P_s$  is the designated chloramphenicol equivalent, in  $\mu\text{g}$  per mg, of USP Chloramphenicol Palmitate RS; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Chloramphenicol Palmitate Oral Suspension

» Chloramphenicol Palmitate Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of chloramphenicol ( $\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_5$ ). It contains one or more suitable buffers, colors, flavors, preservatives, and suspending agents.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Chloramphenicol Palmitate RS



USP Chloramphenicol Palmitate Polymorph A RS

USP Chloramphenicol Palmitate Nonpolymorph A RS

**Identification**—The retention time of the chloramphenicol palmitate peak in the chromatogram of the *Assay preparation* corresponds to that of the chloramphenicol palmitate peak in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Uniformity of dosage units** (905)—

FOR SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 4.5 and 7.0.

**Limit of polymorph A**—

*Standard preparation*—Prepare a dry mixture of 1 part by weight of USP Chloramphenicol Palmitate Polymorph A RS and 9 parts by weight of USP Chloramphenicol Palmitate Nonpolymorph A RS. Prepare a 1 in 3 mineral oil dispersion of this mixture, and place a portion of it between two sodium chloride plates, taking care not to allow air bubbles to form.

*Test preparation*—Place 20 mL of previously mixed Oral Suspension in a 50-mL centrifuge tube, add 20 mL of water, and mix. Centrifuge, and discard the supernatant. Add 20 mL of water to the residue in the centrifuge tube, mix, centrifuge, and discard the supernatant. Repeat this washing two times. Dry the residue in vacuum over silica gel for not less than 14 hours. Prepare a 1 in 3 mineral oil dispersion of the dried residue, and place a portion of it between two sodium chloride plates, taking care not to allow air bubbles to form.

*Procedure*—Concomitantly record the absorption spectra of the *Standard preparation* and the *Test preparation* from about 11  $\mu\text{m}$  to about 13  $\mu\text{m}$ , with a suitable IR absorption spectrophotometer, using an empty cell to set the instrument to 100 percent transmittance. Adjust the cell thickness of the *Standard preparation* and of the *Test preparation* so that transmittances of 20% to 30% are obtained at 12.3  $\mu\text{m}$ . On each spectrum, draw a straight baseline between the absorption minima at wavelengths of about 11.3  $\mu\text{m}$  and 12.65  $\mu\text{m}$ . Draw straight lines, perpendicular to the wavelength scale, at the wavelengths of maximum absorption at about 11.65  $\mu\text{m}$  and 11.86  $\mu\text{m}$ , intersecting both the baseline and the spectrum. Determine the absorbance ratio:

$$(A_{11.65a} - A_{11.65b}) / (A_{11.86a} - A_{11.86b})$$

in which the parenthetical expressions are the differences in absorbance values obtained at the wavelengths indicated by the subscripts for the spectrum (a) and at the point of intersection of the perpendicular line with the baseline (b). The absorbance ratio of the *Test preparation* is greater than that of the *Standard preparation*, corresponding to not more than 10% of polymorph A.

**Assay**—

*Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay under Chloramphenicol Palmitate*.

*Assay preparation*—Transfer an accurately measured volume of Oral Suspension, well-shaken and free from air bubbles, equivalent to about 160 mg of chloramphenicol, to a 200-mL volumetric flask containing about 20 mL of methanol, add 4 mL of glacial acetic acid, dilute with methanol to volume, and mix. Filter about 20 mL of this solution through glass-fiber filter paper. Transfer 10.0 mL of the filtrate to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay under Chloramphenicol Palmitate*. Calculate the quantity, in

mg, of chloramphenicol ( $\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_5$ ) equivalent in each mL of Oral Suspension taken by the formula:

$$0.004(W_s / V)(P_s)(r_u / r_s)$$

in which V is the volume, in mL, of Oral Suspension taken, and the other terms are as defined therein.

## Chloramphenicol Sodium Succinate

$\text{C}_{15}\text{H}_{15}\text{Cl}_2\text{N}_2\text{NaO}_8$  445.18

Butanedioic acid, mono[2-[(2,2-dichloroacetyl)amino]-3-hydroxy-3-(4-nitrophenyl)propyl] ester, monosodium salt, [R-(R\*,R\*)]-.

D-threo-(-)-2,2-Dichloro-N-[[ $\beta$ -hydroxy- $\alpha$ -(hydroxymethyl)-p-nitrophenethyl]acetamide  $\alpha$ -(sodium succinate) [982-57-0].

» Chloramphenicol Sodium Succinate has a potency equivalent to not less than 650  $\mu\text{g}$  and not more than 765  $\mu\text{g}$  of chloramphenicol ( $\text{C}_{11}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_5$ ) per mg.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.

**USP Reference standards** (11)—

USP Chloramphenicol RS

USP Endotoxin RS

**Identification**—The *Assay preparation* exhibits an absorption maximum at a wavelength of about 276 nm, as obtained in the *Assay*.

**Specific rotation** (781S): between +5.0° and +8.0°.

*Test solution*: 50 mg per mL.

**pH** (791): between 6.4 and 7.0, in a solution containing the equivalent of 250 mg of chloramphenicol per mL.

**Water Determination**, *Method I* (921): not more than 5.0%.

**Limit of free chloramphenicol**—

*Mobile phase*—Prepare a filtered and degassed mixture of 0.05 M monobasic ammonium phosphate, previously adjusted with 10% (v/v) phosphoric acid to a pH of  $2.5 \pm 0.1$ , and methanol (60:40). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

*Standard solution*—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in *Mobile phase* to obtain a solution having a known concentration of about 6  $\mu\text{g}$  per mL. Pass this solution through a filter having a 0.5- $\mu\text{m}$  or finer porosity, and use the filtrate.

*Test solution*—Transfer about 33 mg of Chloramphenicol Sodium Succinate, accurately weighed, to a 50-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a filter having a 0.5- $\mu\text{m}$  or finer porosity.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 275-nm detector and a 4.6-mm  $\times$  10-cm column that contains 5- $\mu\text{m}$  packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the two major peaks, chloramphenicol-1-succinate and chloramphenicol-3-succinate, is not less than 1750 theoretical plates; the resolution,  $R$ , between the two peaks is not less than 2.0; and the tailing factor is not more than 1.2. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.



**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the free chloramphenicol peaks. Calculate the percentage of free chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) in the portion of Chloramphenicol Sodium Succinate taken by the formula:

$$5000(C/WQ)(r_U/r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Chloramphenicol RS in the *Standard solution*;  $W$  is the quantity, in mg, of Chloramphenicol Sodium Succinate taken to prepare the *Test solution*;  $Q$  is the quantity, in  $\mu$ g, of chloramphenicol in each mg of Chloramphenicol Sodium Succinate taken, as obtained in the *Assay*; and  $r_U$  and  $r_S$  are the peak areas obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 2.0% is found.

**Other requirements**—Where the label states that Chloramphenicol Sodium Succinate is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Chloramphenicol Sodium Succinate for Injection*. Where the label states that Chloramphenicol Sodium Succinate must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Chloramphenicol Sodium Succinate for Injection*.

#### Assay—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chloramphenicol RS in water, and dilute quantitatively with water to obtain a solution having a known concentration of about 20  $\mu$ g per mL.

**Assay preparation**—Dissolve an accurately weighed quantity of Chloramphenicol Sodium Succinate in water, and dilute quantitatively with water to obtain a solution having a concentration equivalent to about 20  $\mu$ g of chloramphenicol per mL.

**Procedure**—Concomitantly determine the absorbance of the *Standard preparation*, at the wavelength of maximum absorbance at about 278 nm, and the absorbance of the *Assay preparation*, at the wavelength of maximum absorbance at about 276 nm, in 1-cm cells, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in  $\mu$ g, of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) in each mg of Chloramphenicol Sodium Succinate taken by the formula:

$$(CP/W)(A_U/A_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Chloramphenicol RS in the *Standard preparation*;  $P$  is the potency, in  $\mu$ g per mg, of USP Chloramphenicol RS;  $W$  is the weight, in  $\mu$ g, of Chloramphenicol Sodium Succinate taken in each mL of the *Assay preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

### Chloramphenicol Sodium Succinate for Injection

» Chloramphenicol Sodium Succinate for Injection contains an amount of Chloramphenicol Sodium Succinate equivalent to not less than 90.0 percent and not more than 115.0 percent of the labeled amount of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ).

#### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

#### USP Reference standards (11)—

USP Chloramphenicol RS

USP Endotoxin RS

**Bacterial Endotoxins Test** (85)—It contains not more than 0.2 USP Endotoxin Unit per mg of chloramphenicol.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

#### Limit of free chloramphenicol—

*Mobile phase*, *Standard solution*, and *Chromatographic system*—Proceed as directed in the test for *Limit of free chloramphenicol* under *Chloramphenicol Sodium Succinate*.

*Test solution*—Dissolve the contents of 1 container in a volume of *Mobile phase* to obtain a solution containing the equivalent of about 100 mg of chloramphenicol per mL. Dilute this solution quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution containing the equivalent of about 0.5 mg of chloramphenicol per mL. Pass a portion of this solution through a filter having a 0.5- $\mu$ m or finer porosity, and use the filtrate.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the free chloramphenicol peaks. Calculate the percentage of free chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ) in the specimen taken by the formula:

$$0.1(C/D)(r_U/r_S)$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Chloramphenicol RS in the *Standard solution*;  $D$  is the concentration, in mg per mL, of chloramphenicol equivalent in the *Test solution*, based on the labeled quantity in the container and the extent of dilution; and  $r_U$  and  $r_S$  are the chloramphenicol peak areas obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 2.0% is found.

**Other requirements**—It meets the requirements of the tests for *Identification*, *Specific rotation*, *pH*, and *Water* under *Chloramphenicol Sodium Succinate*.

#### Assay—

**Standard preparation**—Proceed as directed in the *Assay* under *Chloramphenicol Sodium Succinate*.

**Assay preparation**—Constitute 1 container of Chloramphenicol Sodium Succinate for Injection as directed in the labeling. Dilute an accurately measured volume of the constituted solution quantitatively with water to obtain a solution having a concentration of about 20  $\mu$ g of chloramphenicol per mL.

**Procedure**—Proceed as directed in the *Assay* under *Chloramphenicol Sodium Succinate*. Calculate the quantity, in mg, of chloramphenicol ( $C_{11}H_{12}Cl_2N_2O_5$ ), in each mL of the constituted Chloramphenicol Sodium Succinate for Injection taken by the formula:

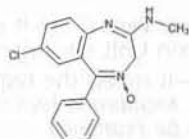
$$(L/D)(CP/1000)(A_U/A_S)$$

in which  $L$  is the labeled quantity, in mg, of chloramphenicol in each mL of constituted solution;  $D$  is the concentration, in  $\mu$ g per mL, of chloramphenicol in the *Assay preparation*, on the basis of the labeled quantity of chloramphenicol in each mL of constituted solution and the extent of dilution;  $C$  is the concentration, in  $\mu$ g per mL, of USP Chloramphenicol RS in the *Standard preparation*;  $P$  is the potency, in  $\mu$ g per mg, of USP Chloramphenicol RS; and  $A_U$  and  $A_S$  are



the absorbances of the Assay preparation and the Standard preparation, respectively.

## Chlordiazepoxide



$C_{16}H_{14}ClN_3O$  299.75

3H-1,4-Benzodiazepin-2-amine, 7-chloro-N-methyl-5-phenyl-, 4-oxide;

7-Chloro-2-(methylamino)-5-phenyl-3H-1,4-benzodiazepine 4-oxide [58-25-3].

### DEFINITION

Chlordiazepoxide contains NLT 98.0% and NMT 102.0% of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (17K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **C.**

**Solution A:** 1 mg/mL of sodium nitrite in water

**Solution B:** 5 mg/mL of ammonium sulfamate in water

**Solution C:** 1 mg/mL of N-(1-naphthyl)ethylenediamine dihydrochloride in water

**Sample:** 20 mg of Chlordiazepoxide

**Analysis:** Add 5 mL of hydrochloric acid and 10 mL of water to the *Sample*, and heat to boiling to effect hydrolysis. Allow the solution to cool. Add 2 mL of *Solution A*, shake, add 1 mL of *Solution B*, shake for 2 min, and add 1 mL of *Solution C*.

**Acceptance criteria:** A reddish-violet color is produced.

### ASSAY

#### PROCEDURE

Use low-actinic glassware.

**Mobile phase:** Methanol and water (60:40)

**Standard solution:** 0.2 mg/mL of USP Chlordiazepoxide RS in *Mobile phase*

**Sample stock solution:** 2 mg/mL of Chlordiazepoxide in *Mobile phase* prepared as follows. Transfer a suitable portion of Chlordiazepoxide to an appropriate volumetric flask, and dissolve in *Mobile phase*. Sonicate for 5 min, and dilute with *Mobile phase* to volume. Pass through a membrane filter of 0.5- $\mu$ m or finer pore size.

**Sample solution:** 0.2 mg/mL of Chlordiazepoxide from *Sample stock solution* in *Mobile phase*

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 5  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 3600 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ) in the portion of Chlordiazepoxide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Chlordiazepoxide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Chlordiazepoxide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

#### Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1, Jan-2018)

#### ORGANIC IMPURITIES

**Standard solution A:** 100  $\mu$ g/mL of USP Chlordiazepoxide Related Compound A RS in acetone

**Standard solution B:** 10  $\mu$ g/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone

**Sample solution:** Transfer 50.0 mg of Chlordiazepoxide to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

#### Chromatographic system

(See Chromatography (621).)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

#### Application volumes

**Standard solution A and Standard solution B:** 10  $\mu$ L

**Sample solution:** 50  $\mu$ L

**Developing solvent system:** Ethyl acetate

**Spray reagent A:** 2 N sulfuric acid

**Spray reagent B:** 1 mg/mL of sodium nitrite in water

**Spray reagent C:** 5 mg/mL of ammonium sulfamate in water

**Spray reagent D:** 1 mg/mL of N-(1-naphthyl)ethylenediamine dihydrochloride in water

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in a chromatographic chamber (not previously saturated with the *Developing solvent system*) using the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent A*, drying at 105° for 15 min, and then spraying in succession with *Spray reagent B*, *Spray reagent C*, and *Spray reagent D*.

**Acceptance criteria:** Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective  $R_f$  values produced by the *Standard solutions*,



corresponding to NMT 0.1% of chlordiazepoxide related compound A, and NMT 0.01% of 2-amino-5-chlorobenzophenone.

### SPECIFIC TESTS

#### • LOSS ON DRYING (731)

Analysis: Dry at 105° for 3 h.  
Acceptance criteria: NMT 0.3%

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in tight, light-resistant containers.

#### • USP REFERENCE STANDARDS (11)

USP 2-Amino-5-chlorobenzophenone RS  
 $C_{13}H_{10}ClNO$  231.68  
USP Chlordiazepoxide RS  
USP Chlordiazepoxide Related Compound A RS  
7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.  
 $C_{15}H_{11}ClN_2O_2$  286.72

## Chlordiazepoxide Tablets

### DEFINITION

Chlordiazepoxide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.**

**Solution A:** 1 mg/mL of sodium nitrite in water  
**Solution B:** 5 mg/mL of ammonium sulfamate in water  
**Solution C:** 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in water  
**Sample:** Nominally 20 mg of chlordiazepoxide from a portion of finely powdered Tablets  
**Analysis:** Add 5 mL of hydrochloric acid and 10 mL of water to the *Sample*, and heat to boiling to effect hydrolysis. Allow the solution to cool. Add 2 mL of *Solution A*, shake, add 1 mL of *Solution B*, shake for 2 min, and add 1 mL of *Solution C*.  
**Acceptance criteria:** A reddish-violet color is produced.

### ASSAY

#### • PROCEDURE

Use low-actinic glassware.

**Mobile phase:** Methanol and water (60:40)

**Standard solution:** 0.2 mg/mL of USP Chlordiazepoxide RS in *Mobile phase*

**Sample solution:** Nominally 0.2 mg/mL of chlordiazepoxide from NLT 20 Tablets prepared as follows. Finely powder NLT 20 Tablets, and transfer a portion of powder equivalent to 5 mg of chlordiazepoxide to a 25-mL volumetric flask. Add 20 mL of *Mobile phase*, sonicate for 5 min, dilute with *Mobile phase* to volume, and pass through a membrane filter of 5- $\mu$ m pore size. Discard the first 5 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 5  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 3600 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Chlordiazepoxide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of chlordiazepoxide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Medium:** Simulated gastric fluid TS, prepared without pepsin; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Standard solution:** USP Chlordiazepoxide RS in *Medium*

**Sample solution:** Pass a portion of solution under test through a suitable filter. Dilute with *Medium*, if necessary.

#### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** The wavelength of maximum absorbance at about 309 nm

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ) dissolved.

**Tolerances:** NLT 85% (Q) of the labeled amount of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

### IMPURITIES

#### • ORGANIC IMPURITIES

**Standard solution A:** 1 mg/mL of USP Chlordiazepoxide Related Compound A RS in acetone

**Standard solution B:** 100  $\mu$ g/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone

**Sample solution:** Transfer a portion of finely powdered Tablets equivalent to 25 mg of chlordiazepoxide to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

#### Chromatographic system

(See *Chromatography* (621).)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

#### Application volumes

**Standard solution A:** 20  $\mu$ L

**Standard solution B:** 5  $\mu$ L

**Sample solution:** 50  $\mu$ L

**Developing solvent system:** Ethyl acetate

**Spray reagent A:** 2 N sulfuric acid

**Spray reagent B:** 1 mg/mL of sodium nitrite in water

**Spray reagent C:** 5 mg/mL of ammonium sulfamate in water



**Spray reagent D:** 1 mg/mL of N-(1-naphthyl)ethylenediamine dihydrochloride in water

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in a chromatographic chamber (not previously saturated with the *Developing solvent system*) in *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent A*, drying at 105° for 15 min, and then spraying in succession with *Spray reagent B*, *Spray reagent C*, and *Spray reagent D*.

**Acceptance criteria:** Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective  $R_f$  values produced by the *Standard solutions*, corresponding to NMT 4.0% of chlordiazepoxide related compound A, and NMT 0.1% of 2-amino-5-chlorobenzophenone.

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP 2-Amino-5-chlorobenzophenone RS

$C_{13}H_{10}ClNO$  231.68

USP Chlordiazepoxide RS

USP Chlordiazepoxide Related Compound A RS

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.

$C_{15}H_{11}ClN_2O_2$  286.72

## Chlordiazepoxide and Amitriptyline Hydrochloride Tablets

#### DEFINITION

Chlordiazepoxide and Amitriptyline Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ) and an amount of amitriptyline hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of amitriptyline ( $C_{20}H_{23}N$ ).

#### IDENTIFICATION

• **A.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

Use low-actinic glassware.

**Buffer:** Combine 10.5 mL of 0.20 N sodium hydroxide and 100 mL of *Solution A*.

**Solution A:** 0.04 M acetic acid, 0.04 M phosphoric acid, and 0.04 M boric acid prepared as follows. Dissolve 2.402 g of glacial acetic acid, 4.612 g of phosphoric acid, and 2.473 g of boric acid in sufficient water to obtain a 1-L solution.

**Diluent:** Methanol, tetrahydrofuran, and *Buffer* (10:40:50)

**Mobile phase:** 0.01 M sodium lauryl sulfate in *Diluent*

**Standard solution:** 0.1 mg/mL of USP Chlordiazepoxide RS and 0.28 mg/mL of USP Amitriptyline Hydrochloride RS in *Diluent*

**Sample stock solution:** Nominally 1 mg/mL of chlordiazepoxide and nominally 2.5 mg/mL of amitriptyline from NLT 20 Tablets prepared as follows. Finely powder NLT 20 Tablets. Transfer a portion of the powder equivalent to 50 mg of chlordiazepoxide and

125 mg of amitriptyline, to a 50-mL volumetric flask. Add *Diluent* to volume, sonicate to disperse the mixture, and allow undissolved particles to settle.

**Sample solution:** Nominally 0.1 mg/mL of chlordiazepoxide and nominally 0.25 mg/mL of amitriptyline from *Sample stock solution* in *Diluent*. Pass a portion of this solution through a filter of 0.5- $\mu$ m or finer pore size. Use the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention times for chlordiazepoxide and amitriptyline are about 5 and 7 min, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between chlordiazepoxide and amitriptyline

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of chlordiazepoxide from the *Sample solution*

$r_S$  = peak response of chlordiazepoxide from the *Standard solution*

$C_S$  = concentration of USP Chlordiazepoxide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of chlordiazepoxide in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of amitriptyline ( $C_{20}H_{23}N$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of amitriptyline from the *Sample solution*

$r_S$  = peak response of amitriptyline from the *Standard solution*

$C_S$  = concentration of USP Amitriptyline Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of amitriptyline in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of amitriptyline, 277.40

$M_{r2}$  = molecular weight of amitriptyline hydrochloride, 313.86

**Acceptance criteria:** 90.0%–110.0% each of the labeled amounts of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ) and amitriptyline ( $C_{20}H_{23}N$ )

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** Simulated gastric fluid TS, prepared without pepsin; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Standard solution A:** USP Chlordiazepoxide RS in *Medium*

**Standard solution B:** USP Amitriptyline Hydrochloride RS in *Medium*

**Sample solution:** Pass a portion of solution under test through a suitable filter. Dilute with *Medium*, if necessary.



**Instrumental conditions**

Mode: UV

Analytical wavelengths: 239 and 309 nm

Blank: Medium

**Analysis**

Samples: Standard solution A, Standard solution B, Sample solution, and Blank

Calculate the percentage of the labeled amount of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times C_{SA} \times D \times V \times (1/L) \times 100$$

 $A_U$  = absorbance of the Sample solution, 309 nm $A_S$  = absorbance of Standard solution A, 309 nm $C_{SA}$  = concentration of USP Chlordiazepoxide RS in Standard solution A (mg/mL) $D$  = dilution factor of the Sample solution, if needed $V$  = volume of Medium, 900 mL $L$  = label claim of chlordiazepoxide (mg/Tablet)Calculate the absorbance of amitriptyline in the Sample solution at 239 nm ( $A_U$ ):

$$\text{Result} = A_{U239} - \{A_{U309} \times [(C_{S309} \times A_{S239}) / (C_{S239} \times A_{S309})]\}$$

 $A_{U239}$  = absorbance of the Sample solution, 239 nm $A_{U309}$  = absorbance of the Sample solution, 309 nm $C_{S309}$  = concentration of chlordiazepoxide from Standard solution A, 309 nm $A_{S239}$  = absorbance of Standard solution A, 239 nm $C_{S239}$  = concentration of chlordiazepoxide from Standard solution A, 239 nm $A_{S309}$  = absorbance of Standard solution A, 309 nmCalculate the percentage of the labeled amount of amitriptyline ( $C_{20}H_{23}N$ ) in the portion of Tablets taken:

$$\text{Result} = (A_X/A_S) \times C_S \times D \times (M_{r1}/M_{r2}) \times V \times (1/L) \times 100$$

 $A_X$  = absorbance of the Sample solution determined from the previous equation $A_S$  = absorbance of amitriptyline from Standard solution B, 239 nm $C_S$  = concentration of USP Amitriptyline Hydrochloride RS in Standard solution B (mg/mL) $D$  = dilution factor of the Sample solution, if needed $M_{r1}$  = molecular weight of amitriptyline, 277.40 $M_{r2}$  = molecular weight of amitriptyline hydrochloride, 313.86 $V$  = volume of Medium, 900 mL $L$  = label claim of amitriptyline (mg/Tablet)

[NOTE—All of the chlordiazepoxide measurements may be made with either a single Standard solution or two separate Standard solutions.]

**Tolerances:** NLT 85% (Q) of the labeled amount of chlordiazepoxide ( $C_{16}H_{14}ClN_3O$ ), and an amount of amitriptyline hydrochloride equivalent to NLT 85% (Q) of the labeled amount of amitriptyline ( $C_{20}H_{23}N$ ) are dissolved.

- UNIFORMITY OF DOSAGE UNITS, Content Uniformity (905):** Meet the requirements for both chlordiazepoxide and amitriptyline

**IMPURITIES****• ORGANIC IMPURITIES**

Standard solution A: 1 mg/mL of USP Chlordiazepoxide Related Compound A RS in acetone

Standard solution B: 50 µg/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone

Sample solution: Transfer a portion of finely powdered Tablets equivalent to 25 mg of chlordiazepoxide to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

**Chromatographic system**

(See Chromatography (621).)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel

**Application volumes**

Standard solution A: 20 µL

Standard solution B: 10 µL

Sample solution: 50 µL

Developing solvent system: Ethyl acetate

Spray reagent A: 2 N sulfuric acid

Spray reagent B: 1 mg/mL of sodium nitrite in water

Spray reagent C: 5 mg/mL of ammonium sulfamate in water

Spray reagent D: 1 mg/mL of N-(1-naphthyl)ethylenediamine dihydrochloride in water

**Analysis**

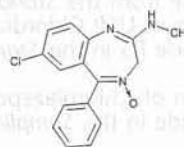
Samples: Standard solution A, Standard solution B, and Sample solution

Develop the chromatogram in a chromatographic chamber (not previously saturated with the Developing solvent system) using the Developing solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with Spray reagent A, drying at 105° for 15 min, and then spraying in succession with Spray reagent B, Spray reagent C, and Spray reagent D.

**Acceptance criteria:** Any spots from the Sample solution are not greater in size or intensity than the spots at the respective  $R_f$  values produced by the Standard solutions, corresponding to NMT 4.0% of chlordiazepoxide related compound A, and NMT 0.1% of 2-amino-5-chlorobenzophenone.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- USP REFERENCE STANDARDS (11)**
  - USP 2-Amino-5-chlorobenzophenone RS  
 $C_{13}H_{10}ClNO$  231.68
  - USP Amitriptyline Hydrochloride RS
  - USP Chlordiazepoxide RS
  - USP Chlordiazepoxide Related Compound A RS
  - 7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.  
 $C_{15}H_{11}ClN_2O_2$  286.72

**Chlordiazepoxide Hydrochloride**

$C_{16}H_{14}ClN_3O \cdot HCl$  336.22  
 3H-1,4-Benzodiazepin-2-amine, 7-chloro-N-methyl-5-phenyl-, 4-oxide, monohydrochloride;  
 7-Chloro-2-(methylamino)-5-phenyl-3H-1,4-benzodiazepine 4-oxide monohydrochloride [438-41-5].

**DEFINITION**

Chlordiazepoxide Hydrochloride contains NLT 98.0% and NMT 102.0% of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ), calculated on the dried basis.



**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C.**
  - Solution A:* 1 mg/mL of sodium nitrite in water
  - Solution B:* 5 mg/mL ammonium sulfamate in water
  - Solution C:* 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in water
  - Sample:* 20 mg of Chlordiazepoxide Hydrochloride
  - Analysis:* Add 5 mL of hydrochloric acid and 10 mL of water to the *Sample*, and heat to boiling to effect hydrolysis. Allow the solution to cool. Add 2 mL of *Solution A*, 1 mL of *Solution B*, and 1 mL of *Solution C*.
  - Acceptance criteria:* A reddish-violet color is produced.

**ASSAY**• **PROCEDURE**

Use low-actinic glassware.

*Mobile phase:* Methanol and water (60:40)

*Standard solution:* 0.2 mg/mL of USP Chlordiazepoxide Hydrochloride RS in *Mobile phase*

*Sample stock solution:* 2 mg/mL of Chlordiazepoxide Hydrochloride in *Mobile phase* prepared as follows.

Transfer a suitable portion of Chlordiazepoxide Hydrochloride to an appropriate volumetric flask, and dissolve in *Mobile phase*. Sonicate for 5 min, and dilute with *Mobile phase* to volume. Pass through a membrane filter of 0.5-μm or finer pore size.

*Sample solution:* 0.2 mg/mL of Chlordiazepoxide Hydrochloride from *Sample stock solution* in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

*Mode:* LC

*Detector:* UV 254 nm

*Column:* 3.9-mm × 30-cm; packing L1

*Flow rate:* 1 mL/min

*Injection volume:* 5 μL

**System suitability**

*Sample:* *Standard solution*

**Suitability requirements**

*Column efficiency:* NLT 3600 theoretical plates

*Tailing factor:* NMT 2.0

*Relative standard deviation:* NMT 2.0%

**Analysis**

*Samples:* *Standard solution* and *Sample solution*

Calculate the percentage of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) in the portion of Chlordiazepoxide Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Chlordiazepoxide Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Chlordiazepoxide Hydrochloride in the *Sample solution* (mg/mL)

*Acceptance criteria:* 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 0.1%

**Delete the following:**

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1: Jan-2018)

• **ORGANIC IMPURITIES**

*Standard solution A:* 100 μg/mL of USP Chlordiazepoxide Related Compound A RS in acetone

*Standard solution B:* 10 μg/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone

*Sample solution:* Transfer 50.0 mg of Chlordiazepoxide Hydrochloride to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

**Chromatographic system**

(See *Chromatography* (621).)

*Mode:* TLC

*Adsorbent:* 0.25-mm layer of chromatographic silica gel

**Application volumes**

*Standard solution A and Standard solution B:* 10 μL

*Sample solution:* 50 μL

*Developing solvent system:* Ethyl acetate

*Spray reagent A:* 2 N sulfuric acid

*Spray reagent B:* 1 mg/mL of sodium nitrite in water

*Spray reagent C:* 5 mg/mL of ammonium sulfamate in water

*Spray reagent D:* 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in water

**Analysis**

*Samples:* *Standard solution A*, *Standard solution B*, and *Sample solution*

Develop the chromatogram in a chromatographic chamber (not previously saturated with the *Developing solvent system*) using the *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent A*, drying at 105° for 15 min, and then spraying in succession with *Spray reagent B*, *Spray reagent C*, and *Spray reagent D*.

*Acceptance criteria:* Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective  $R_f$  values produced by the *Standard solutions*, corresponding to NMT 0.1% of chlordiazepoxide related compound A, and NMT 0.01% of 2-amino-5-chlorobenzophenone.

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE, Class I (741):**

212°–218°, with decomposition

- **LOSS ON DRYING (731)**

*Analysis:* Dry under vacuum over phosphorus pentoxide at 60° for 4 h.

*Acceptance criteria:* NMT 0.5%

- **STERILITY TESTS (71):** Where the label states that

Chlordiazepoxide Hydrochloride is sterile, it meets the requirements

- **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Chlordiazepoxide Hydrochloride is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 3.57 USP Endotoxin Units/mg of Chlordiazepoxide Hydrochloride.

- **OTHER REQUIREMENTS:** Where the label states that Chlordiazepoxide Hydrochloride is sterile, it meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

- **USP REFERENCE STANDARDS (11)**

USP 2-Amino-5-chlorobenzophenone RS  
 $C_{13}H_{10}ClNO$  231.68



USP Chlordiazepoxide Hydrochloride RS  
 USP Chlordiazepoxide Related Compound A RS  
 7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-  
 2-one 4-oxide.  
 $C_{15}H_{11}ClN_2O_2$  286.72  
 USP Endotoxin RS

## Chlordiazepoxide Hydrochloride Capsules

### DEFINITION

Chlordiazepoxide Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of chlordiazepoxide hydrochloride ( $C_{15}H_{11}ClN_2O_2 \cdot HCl$ ).

### IDENTIFICATION

#### A.

**Diluent:** Sulfuric acid in dehydrated alcohol (1 in 360)  
**Sample stock solution:** Nominally 0.6 mg/mL of chlordiazepoxide hydrochloride from Capsule powder prepared as follows. Transfer a portion of the Capsule powder, equivalent to 60 mg of chlordiazepoxide hydrochloride, to a 100-mL flask. Add methanol to volume, and filter, discarding the first 15 mL of the filtrate. Use the clear filtrate.

**Sample solution:** Nominally 6 µg/mL of chlordiazepoxide hydrochloride from *Sample stock solution* in *Diluent*

#### Instrumental conditions

**Mode:** UV  
**Cell:** 1 cm  
**Blank:** *Diluent*

#### Analysis

**Sample:** *Sample solution*

**Acceptance criteria:** The *Sample solution* exhibits maxima at  $245 \pm 2$  nm and  $311 \pm 2$  nm, and the ratio  $A_{245}/A_{311}$  is between 2.90 and 3.45.

#### B.

**Solution A:** 1 mg/mL of sodium nitrite in water  
**Solution B:** 5 mg/mL of ammonium sulfamate in water  
**Solution C:** 1 mg/mL of *N*-(1-naphthyl)ethylenediamine dihydrochloride in water

**Sample:** A portion of the contents of Capsules

**Analysis:** Add 5 mL of hydrochloric acid and 10 mL of water to the *Sample*, and heat to boiling to effect hydrolysis. Allow the solution to cool. Add 2 mL of *Solution A*, 1 mL of *Solution B*, and 1 mL of *Solution C*.

**Acceptance criteria:** A reddish-violet color is produced.

### ASSAY

#### PROCEDURE

Use low-actinic glassware.

**Mobile phase:** Methanol and water (60:40)

**Standard solution:** 0.2 mg/mL of USP Chlordiazepoxide Hydrochloride RS in *Mobile phase*

**Sample solution:** Nominally 0.2 mg/mL of chlordiazepoxide hydrochloride from the contents of Capsules prepared as follows. Combine the contents of NLT 20 Capsules in a suitable container, and transfer a portion of the contents, equivalent to 5 mg of chlordiazepoxide hydrochloride, to a 25-mL volumetric flask. Add 20 mL of *Mobile phase*, sonicate for 5 min, dilute with *Mobile phase* to volume, and pass through a membrane filter of 5-µm pore size. Discard the first 5 mL of the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 5 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 3600 theoretical plates

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlordiazepoxide hydrochloride ( $C_{15}H_{11}ClN_2O_2 \cdot HCl$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Chlordiazepoxide Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of chlordiazepoxide hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION (711)

**Medium:** Water; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Standard solution:** USP Chlordiazepoxide Hydrochloride RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

#### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** The wavelength of maximum absorbance at about 245 nm

**Cell:** 1 cm

**Blank:** Remove the contents of 12 Capsules as completely as possible with the aid of a current of air. Dissolve the empty Capsule shells in 900 mL of *Medium*. Pass a portion of the resulting solution through a suitable filter. Dilute with *Medium*, if necessary, for consistency with the treatment of the *Sample solution*.

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*

Determine the percentage of the labeled amount of chlordiazepoxide hydrochloride ( $C_{15}H_{11}ClN_2O_2 \cdot HCl$ ) dissolved.

**Tolerances:** NLT 85% (Q) of the labeled amount of chlordiazepoxide hydrochloride ( $C_{15}H_{11}ClN_2O_2 \cdot HCl$ ) is dissolved.

#### UNIFORMITY OF DOSAGE UNITS (905)

##### Procedure for content uniformity

Use low-actinic glassware.

**Diluent:** 0.1 N hydrochloric acid

**Standard solution:** 6 µg/mL of USP Chlordiazepoxide Hydrochloride RS in *Diluent*

**Sample stock solution:** Transfer the contents of 1 Capsule to a 200-mL volumetric flask, dissolve in water, and dilute with water to volume. Filter and discard the first 20 mL of the filtrate.

**Sample solution:** Nominally 6 µg/mL of chlordiazepoxide hydrochloride from *Sample stock solution* in *Diluent*

#### Instrumental conditions

**Mode:** UV-Vis

**Analytical wavelength:** The wavelength of maximum absorbance at about 245 nm



Cell: 1 cm  
Blank: Diluent

**Analysis**

**Samples:** Standard solution, Sample solution, and Blank

Concomitantly determine the absorbances of the Standard solution and the Sample solution using the Blank. Calculate the percentage of the labeled amount of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) in the Capsule taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- $A_U$  = absorbance of the Sample solution  
 $A_S$  = absorbance of the Standard solution  
 $C_S$  = concentration of USP Chlordiazepoxide Hydrochloride RS in the Standard solution ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of chlordiazepoxide hydrochloride in the Sample solution ( $\mu\text{g/mL}$ )  
**Acceptance criteria:** Meet the requirements

**IMPURITIES**• **ORGANIC IMPURITIES**

**Standard solution A:** 1 mg/mL of USP Chlordiazepoxide Related Compound A RS in acetone

**Standard solution B:** 0.05 mg/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone

**Sample solution:** Transfer a portion of Capsule contents equivalent to 25 mg of chlordiazepoxide hydrochloride to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

**Chromatographic system**

(See *Chromatography* (621).)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volumes**

**Standard solution A:** 15  $\mu\text{L}$

**Standard solution B:** 10  $\mu\text{L}$

**Sample solution:** 50  $\mu\text{L}$

**Developing solvent system:** Ethyl acetate

**Spray reagent A:** 2 N sulfuric acid

**Spray reagent B:** 1 mg/mL of sodium nitrite in water

**Spray reagent C:** 5 mg/mL of ammonium sulfamate in water

**Spray reagent D:** 1 mg/mL of N-(1-naphthyl)ethylenediamine dihydrochloride in water

**Analysis**

**Samples:** Standard solution A, Standard solution B, and Sample solution

Develop the chromatogram in a chromatographic chamber (not previously saturated with the Developing solvent system) using the Developing solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with Spray reagent A, drying at 105° for 15 min, and then spraying in succession with Spray reagent B, Spray reagent C, and Spray reagent D.

**Acceptance criteria:** Any spots from the Sample solution are not greater in size or intensity than the spots at the respective  $R_f$  values produced by the Standard solutions, corresponding to NMT 3.0% of chlordiazepoxide related compound A, and NMT 0.1% of 2-amino-5-chlorobenzophenone.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP 2-Amino-5-chlorobenzophenone RS  
 $C_{13}H_{10}ClNO$  231.68

USP Chlordiazepoxide Hydrochloride RS

USP Chlordiazepoxide Related Compound A RS

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.

$C_{15}H_{11}ClN_2O_2$  286.72

## Chlordiazepoxide Hydrochloride and Clidinium Bromide Capsules

**DEFINITION**

Chlordiazepoxide Hydrochloride and Clidinium Bromide Capsules contain NLT 90.0% and NMT 110.0% of the labeled amounts of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) and clidinium bromide ( $C_{22}H_{26}BrNO_3$ ).

**IDENTIFICATION**

- **A.** The retention times of the major peaks of the Sample solution correspond to those of the Standard solution, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

[NOTE—Use low-actinic glassware.]

**Buffer:** Dissolve 1.92 g of sodium 1-pentanesulfonate in 900 mL of water in a 1-L volumetric flask. Adjust with 1 N sulfuric acid to a pH of  $3.8 \pm 0.1$ . Dilute with water to volume.

**Mobile phase:** Methanol, tetrahydrofuran, and Buffer (6:24:70)

**Diluent:** Methanol and water (1:1)

**Standard solution:** 0.1 mg/mL of USP Chlordiazepoxide Hydrochloride RS and 0.05 mg/mL of USP Clidinium Bromide RS in Diluent

**Sample solution:** Weigh the contents of NLT 20 Capsules, and calculate the average weight per Capsule. Mix the combined contents of the Capsules, and transfer an amount equivalent to about 5 mg of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) to a 50-mL volumetric flask. Add about 25 mL of Diluent, sonicate for 5 min, and shake by mechanical means for 10 min. Dilute with Diluent to volume, and filter, discarding the first 20 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 212 nm

**Column:** 8-mm  $\times$  10-cm; packing L1

**Flow rate:** 3 mL/min

**Injection size:** 20  $\mu\text{L}$

**System suitability**

**Sample:** Standard solution

[NOTE—The relative retention times for clidinium bromide and chlordiazepoxide hydrochloride are about 0.5 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 5.0 between the clidinium bromide and chlordiazepoxide hydrochloride peaks

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of chlordiazepoxide hydrochloride from the Sample solution  
 $r_S$  = peak response of chlordiazepoxide hydrochloride from the Standard solution



$C_S$  = concentration of USP Chlordiazepoxide Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of chlordiazepoxide hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of clidinium bromide ( $C_{22}H_{26}BrNO_3$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clidinium bromide from the *Sample solution*

$r_S$  = peak response of clidinium bromide from the *Standard solution*

$C_S$  = concentration of USP Clidinium Bromide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clidinium bromide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION, Procedure for a Pooled Sample (711)

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Buffer: Dissolve 1.92 g of sodium 1-pentanesulfonate in 900 mL of water in a 1-L volumetric flask. Adjust with dilute sulfuric acid to a pH of  $3.8 \pm 0.1$ . Dilute with water to volume.

Mobile phase: Methanol, tetrahydrofuran, and Buffer (6:18:75)

Standard solution: Prepare a solution having known concentrations of USP Chlordiazepoxide Hydrochloride RS and USP Clidinium Bromide RS in *Medium*.

Sample solution: Pass a portion of the solution under test through a suitable filter. Combine equal volumes of the filtered solutions and use the pooled sample for the analysis. Dilute with *Medium* to a concentration that is similar to that of the *Standard solution*, if necessary.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 212 nm

Column: 4-mm  $\times$  25-cm; packing L1

Flow rate: 2 mL/min

Injection size: 100  $\mu$ L

### System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for clidinium bromide and chlordiazepoxide hydrochloride are about 0.6 and 1.0, respectively.]

### Suitability requirements

Resolution: NLT 5.0 between the clidinium bromide and chlordiazepoxide hydrochloride peaks

Relative standard deviation: NMT 2.0%

### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the average percentage of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) or clidinium bromide ( $C_{22}H_{26}BrNO_3$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response of chlordiazepoxide hydrochloride or clidinium bromide from the *Sample solution*

$r_S$  = peak response of chlordiazepoxide hydrochloride or clidinium bromide from the *Standard solution*

$C_S$  = concentration of USP Chlordiazepoxide Hydrochloride RS or USP Clidinium Bromide RS in the *Standard solution* (mg/mL)

$L$  = chlordiazepoxide hydrochloride or clidinium bromide label claim (mg)

$V$  = volume of *Medium* (mL), 900

Tolerances: NLT 75% (Q) each of the labeled amounts of chlordiazepoxide hydrochloride ( $C_{16}H_{14}ClN_3O \cdot HCl$ ) and clidinium bromide ( $C_{22}H_{26}BrNO_3$ ) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### • LIMIT OF CHLORDIAZEPOXIDE RELATED COMPOUND A AND 2-AMINO-5-CHLOROBENZOPHENONE

Standard solution A: 1 mg/mL of USP Chlordiazepoxide Related Compound A RS in acetone

Standard solution B: 50  $\mu$ g/mL of USP 2-Amino-5-chlorobenzophenone RS in acetone

Sample solution: Transfer an amount equivalent to 25 mg of chlordiazepoxide hydrochloride from Capsule contents to a 10-mL conical flask, add 2.5 mL of acetone, and shake. Allow any undissolved particles to settle, and use the supernatant.

### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

Adsorbent: 0.25-mm layer of chromatographic silica gel

Application volume: 50  $\mu$ L for the *Sample solution*, 15  $\mu$ L for *Standard solution A*, and 10  $\mu$ L for *Standard solution B*

Developing solvent system: Ethyl acetate

Spray reagent: 2 N sulfuric acid

### Analysis

Samples: *Standard solutions* and *Sample solution*

Proceed as directed in the chapter. Develop the chromatogram in a chromatographic chamber (not previously saturated with the developing solvent) in the *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent*. Dry at 105° for 15 min, and then spray in succession with sodium nitrite solution (1 in 1000), ammonium sulfamate solution (1 in 200), and N-(1-naphthyl)ethylenediamine dihydrochloride solution (1 in 1000).

Acceptance criteria: Any spots from the *Sample solution* are not greater in size or intensity than the spots at the respective  $R_f$  values produced by the *Standard solutions*, corresponding to NMT 3.0% of chlordiazepoxide related compound A and to NMT 0.1% of 2-amino-5-chlorobenzophenone.

### • LIMIT OF CLIDINIUM BROMIDE RELATED COMPOUND A

Extracting solvent mixture: Dehydrated alcohol and cyclohexane (1:1)

Identification solution: Dissolve 50 mg of USP Clidinium Bromide RS in 1 mL of 0.1 N methanolic hydrochloric acid. To this solution add 20  $\mu$ L of a solution of 25 mg/mL of USP Clidinium Bromide Related Compound A RS in methanol. Prepare this solution at the time of use.

Standard solution: 50 mg/mL of USP Clidinium Bromide RS in 0.1 N methanolic hydrochloric acid. [NOTE—Prepare this solution at the time of use.]

Sample solution: Empty a number of Capsules, equivalent to 25 mg of clidinium bromide, into a glass-stoppered centrifuge tube, and add 5 mL of the *Extracting solvent mixture*. Heat the tube gently, with shaking, to 50°, centrifuge, and decant the clear supernatant into a second tube. Repeat the addition of *Extracting solvent mixture* twice, heating, centrifuging, and decanting as before, and combine the three extracts in a single tube. Gently heating, evaporate the combined extracts under a stream of nitrogen to dryness. Dissolve the residue in 0.5 mL of methanol.



**Chromatographic system**

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 20  $\mu$ L

**Developing solvent system:** Acetone, methanol, water, and hydrochloric acid (70:20:5:5)

**Spray reagent:** Dissolve 850 mg of bismuth subnitrate in a mixture of 10 mL of glacial acetic acid and 40 mL of water. In a separate container, dissolve 20 g of potassium iodide in 50 mL of water. Mix the two solutions, and dilute with dilute sulfuric acid (1 in 10) to 500 mL. Add  $7.5 \pm 2.5$  g of iodine, and mix until solution is complete.

**Chromatographic plates:** Predevelop suitable thin-layer chromatographic plates by placing in a chromatographic chamber saturated with *Developing solvent system*, and allow the *Developing solvent system* to move 15 cm. Remove the plates from the chamber, dry at 105° for 15 min, and cool.

**Analysis**

**Samples:** *Identification solution*, *Standard solution*, and *Sample solution*

Proceed as directed in the chapter. Place the plates in an unsaturated chromatographic chamber containing freshly prepared *Developing solvent system*, and develop the chromatogram until the solvent front has moved 15 cm. Remove the plates, and dry at 105° for 10 min. Cool to room temperature, and spray with *Spray reagent*. Any spot in the chromatogram of the *Sample solution* occurring at an  $R_f$  value of 0.4 is not greater in size or intensity than the corresponding spot in the chromatogram of the *Identification solution*; and the *Standard solution* shows no spot at the  $R_f$  value corresponding to that of clidinium bromide related compound A.

**Acceptance criteria:** NMT 1.0% of clidinium bromide related compound A

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

• **USP REFERENCE STANDARDS (11)**

USP 2-Amino-5-chlorobenzophenone RS

2-Amino-5-chlorobenzophenone.

$C_{13}H_{10}ClNO$  231.68

USP Chlordiazepoxide Hydrochloride RS

USP Chlordiazepoxide Related Compound A RS

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one 4-oxide.

$C_{15}H_{11}ClN_2O_2$  286.72

USP Clidinium Bromide RS

USP Clidinium Bromide Related Compound A RS

3-Hydroxy-1-methylquinuclidinium bromide.

$C_8H_{16}BrNO$  222.13

1,1'-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] diacetate [56-95-1].

**DEFINITION**

Chlorhexidine Acetate contains NLT 98.0% and NMT 102.0% of chlorhexidine acetate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$ ), calculated on the dried basis.

**IDENTIFICATION**

• **A. INFRARED ABSORPTION (197K)**

**ASSAY**

• **PROCEDURE**

**Solution A:** 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Prepare a mixture of acetonitrile and this solution (3:7).

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

**System suitability solution:** 50  $\mu$ g/mL of USP Chlorhexidine Acetate RS and 1  $\mu$ g/mL of USP *p*-Chloroaniline RS in *Solution A*

**Standard solution:** 50  $\mu$ g/mL of USP Chlorhexidine Acetate RS in *Solution A*

**Sample solution:** 50  $\mu$ g/mL of Chlorhexidine Acetate in *Solution A*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm  $\times$  25-cm; base-deactivated 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 50  $\mu$ L

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between chlorhexidine and *p*-chloroaniline

**Relative standard deviation:** NMT 0.73% for chlorhexidine and NMT 5.0% for *p*-chloroaniline

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of chlorhexidine acetate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$ ) in the portion of Chlorhexidine Acetate taken:

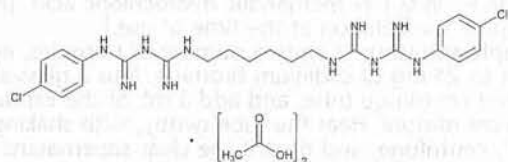
$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of chlorhexidine from the *Sample solution*

$r_S$  = peak response of chlorhexidine from the *Standard solution*

$C_S$  = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = concentration of Chlorhexidine Acetate in the *Sample solution* ( $\mu$ g/mL)

**Chlorhexidine Acetate**

$C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$

2,4,11,13-Tetraazatetradecanediimidamide, *N,N'*-bis(4-chlorophenyl)-3,12-diimino-, diacetate;

625.55



Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.15%

• **ORGANIC IMPURITIES**

Store the *System suitability solution*, the *Sample solution*, and the *Diluted sample solution* at a temperature of NMT 12°.

**Solution A:** 0.1% (v/v) Trifluoroacetic acid in acetonitrile

**Solution B:** 0.1% (v/v) Trifluoroacetic acid in water

**Solution C:** *Solution A* and *Solution B* (20:80)

**Solution D:** *Solution A* and *Solution B* (90:10)

**Mobile phase:** See *Table 2*. Return to original conditions, and equilibrate the system.

**Table 2**

Time (min)	Solution C (%)	Solution D (%)
0	100	0
2	100	0
32	80	20
37	80	20
47	70	30
54	70	30

**System suitability solution:** 5.0 mg/mL of USP Chlorhexidine System Suitability Mixture RS in *Solution C*. See *Table 3* for relative retention times of the main components of the mixture.

**Table 3**

Components of USP Chlorhexidine System Suitability Mixture RS	Relative Retention Time
Chlorhexidine oxazinone analog	0.23
Chlorhexidine amine	0.25
Chlorhexidine guanidine	0.35
Chlorhexidine urea	0.36
<i>p</i> -Chlorophenyl urea	0.5
Chlorhexidine nitrile	0.6
Chlorhexidine dimer	0.85
<i>o</i> -Chlorhexidine	0.90
Specified unidentified impurity 2	0.91
Chlorhexidine glucityl triazine	0.96
Chlorhexidine	1.0
Oxochlorhexidine	1.4

**Sample solution:** 1.4 mg/mL of Chlorhexidine Acetate in *Solution C*

**Diluted sample solution:** Dilute 1.0 mL of *Sample solution* with *Solution C* to 100.0 mL.

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; base-deactivated 5-μm packing L1

**Temperatures**

**Column:** 30°

**Autosampler:** NMT 12°

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Peak-to-valley ratio:** NLT 2.0 between chlorhexidine urea and chlorhexidine guanidine

### Analysis

**Samples:** *Sample solution* and *Diluted sample solution*  
Calculate the percentage of each impurity in the portion of Chlorhexidine Acetate taken:

$$\text{Result} = (r_u/r_s) \times D \times 100$$

$r_u$  = peak response of each impurity from the *Sample solution*

$r_s$  = peak response of chlorhexidine from the *Diluted sample solution*

$D$  = dilution factor used to prepare the *Diluted sample solution*, 0.01

**Acceptance criteria:** See *Table 4*. The reporting level for impurities is 0.05%.

**Table 4**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorhexidine guanidine	0.35	0.15
Chlorhexidine nitrile	0.6	0.15
Chlorhexidine dimer	0.85	0.15
<i>o</i> -Chlorhexidine and specified unidentified impurity 2 <sup>a</sup>	0.90–0.91	0.3 <sup>a</sup>
Chlorhexidine	1.0	—
Oxochlorhexidine	1.4	0.3
Any individual unspecified impurity	—	0.10
Total impurities	—	0.7

<sup>a</sup> If present, *o*-chlorhexidine and specified unidentified impurity 2 may not be completely resolved by the method. These peaks are integrated together to determine conformance.

• **LIMIT OF *p*-CHLOROANILINE**

*Solution A*, *Solution B*, *Mobile phase*, *System suitability solution*, *Chromatographic system*, and *System suitability*: Proceed as directed in the *Assay*.

**Standard solution:** 1.0 μg/mL of USP *p*-Chloroaniline RS in *Solution A*

**Sample solution:** 2.0 mg/mL of Chlorhexidine Acetate in *Solution A*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the amount, in ppm, of *p*-chloroaniline in the portion of Chlorhexidine Acetate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 0.001 \times 10^6$$

$r_u$  = peak response of *p*-chloroaniline from the *Sample solution*

$r_s$  = peak response of *p*-chloroaniline from the *Standard solution*

$C_s$  = concentration of *p*-chloroaniline in the *Standard solution* (μg/mL)

$C_u$  = concentration of Chlorhexidine Acetate in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 500 ppm

### SPECIFIC TESTS

• **LOSS ON DRYING** (731)

**Analysis:** Dry at 105° to constant weight.

**Acceptance criteria:** NMT 3.5%

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

• **USP REFERENCE STANDARDS** (11)

USP Chlorhexidine Acetate RS

USP Chlorhexidine System Suitability Mixture RS

The mixture contains chlorhexidine and the following impurities (other impurities may also be present):



Chlorhexidine oxazinone analog;  
(5*R*,6*S*)-2-[(4-chlorophenyl)amino]-5-hydroxy-6-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-5,6-dihydro-4*H*-1,3-oxazin-4-one.

$C_{13}H_{15}ClN_2O_6$  330.72

Chlorhexidine amine;

1-(6-Aminoheptyl)-5-(4-chlorophenyl)biguanide.

$C_{14}H_{23}ClN_6$  310.83

Chlorhexidine guanidine;

1-[6-(Carbamimidoylamino)hexyl]-5-(4-chlorophenyl)biguanide.

$C_{15}H_{25}ClN_8$  352.87

Chlorhexidine urea;

*N*-[6-[(4-chlorophenyl)carbamimidoyl]-carbamimidoyl]amino)hexyl]carbamimidoyl]urea.

$C_{16}H_{26}ClN_9O$  395.89

*p*-Chlorophenyl urea;

1-(4-chlorophenyl)urea.

$C_7H_7ClN_2O$  170.60

Chlorhexidine nitrile;

1-(4-chlorophenyl)-5-[6-[(cyanocarbamidoyl)-amino]hexyl]biguanide.

$C_{16}H_{24}ClN_9$  377.88

Chlorhexidine dimer;

1,5-Bis[5-(4-chlorophenyl)biguanidylhexyl]biguanide.

$C_{30}H_{47}Cl_2N_{15}$  688.70

*o*-Chlorhexidine;

1-(2-chlorophenyl)-5-[6-[(4-chlorophenyl)carbamimidoyl]carbamimidoyl]amino)-hexyl]biguanide.

$C_{22}H_{30}Cl_2N_{10}$  505.45

Specified unidentified impurity 2;

Chlorhexidine glucityl triazine;

1-(4-chlorophenyl)-5-[6-[(4-chlorophenyl)amino]-6-[(1*S*,2*R*,3*R*,4*R*)-1,2,3,4,5-pentahydroxypentyl]-1,3,5-triazin-2-yl]amino)hexyl]biguanide.

$C_{28}H_{38}Cl_2N_{10}O_5$  665.57

Oxochlorhexidine;

*N*-(4-chlorophenyl)-*N'*-[6-[(4-chlorophenyl)carbamimidoyl]carbamimidoyl]amino)-hexyl]carbamimidoyl]urea.

$C_{22}H_{29}Cl_2N_9O$  506.43

USP *p*-Chloroaniline RS

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

**System suitability solution:** 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

**Standard solution:** 40 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*

**Sample solution:** Nominally 40 µg/mL of chlorhexidine acetate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 20 mg of chlorhexidine acetate, to a 100-mL volumetric flask, and dilute with methanol to volume. Further dilute a 10-mL portion of this solution with *Solution A* to 50 mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 50 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The approximate relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between chlorhexidine and *p*-chloroaniline

**Relative standard deviation:** NMT 2.0% for the chlorhexidine peak, NMT 5.0% for the *p*-chloroaniline peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$  in the portion of Topical Solution taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak area of chlorhexidine from the *Sample solution*

$r_s$  = peak area of chlorhexidine from the *Standard solution*

$C_s$  = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)

$C_u$  = nominal concentration of chlorhexidine acetate in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### IMPURITIES

##### Organic Impurities

##### • PROCEDURE: LIMIT OF *p*-CHLOROANILINE

**Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 1.0 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

**Sample solution:** Nominally 2.0 mg/mL of chlorhexidine acetate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 200 mg of chlorhexidine acetate, to a 100-mL volumetric flask, and dilute with *Solution A* to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** The *p*-chloroaniline peak area from the *Sample solution* is NMT the *p*-chloroaniline

## Chlorhexidine Acetate Topical Solution

### DEFINITION

Chlorhexidine Acetate Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorhexidine acetate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_2H_4O_2$ ).

### IDENTIFICATION

- A.** The retention time of the major peak for chlorhexidine from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- B. IDENTIFICATION TESTS—GENERAL, Acetate (191):** Meets the requirements of the lanthanum nitrate test.  
**Sample:** Evaporate or dilute a volume of Topical Solution containing the equivalent of about 5 mg of chlorhexidine acetate to about 5 mL.

### ASSAY

#### • PROCEDURE

**Solution A:** Dissolve 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Prepare a mixture of the resulting solution and acetonitrile (70:30).

**Solution B:** Acetonitrile

**Mobile phase:** See the gradient table below.



peak area from the *Standard solution* (NMT 500 ppm, calculated with reference to the nominal content of chlorhexidine acetate).

#### SPECIFIC TESTS

- **pH** (791): 5.0–7.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light.
- **LABELING:** Label it to indicate that it is for veterinary use only.
- **USP REFERENCE STANDARDS** (11)
  - USP Chlorhexidine Acetate RS
  - USP *p*-Chloroaniline RS

### Chlorhexidine Gluconate Oral Rinse

» Chlorhexidine Gluconate Oral Rinse is prepared from Chlorhexidine Gluconate Solution. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chlorhexidine gluconate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ ).

**Packaging and storage**—Preserve in tight containers, protected from light, at controlled room temperature.

**Labeling**—Oral Rinse intended solely for veterinary use is so labeled. Oral Rinse intended for human use is labeled to indicate it is to be expectorated and not swallowed after rinsing.

#### USP Reference standards (11)—

- USP Chlorhexidine Acetate RS
- USP *p*-Chloroaniline RS
- USP Potassium Gluconate RS

#### Identification—

**A:** The retention time of the major peak for chlorhexidine in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** To a volume of Oral Rinse, equivalent to about 10 mg of chlorhexidine gluconate, add 5 mL of a solution of cetyltrimethylammonium bromide (1 in 100), 1 mL of 10 N sodium hydroxide, and 1 mL of bromine TS: a deep red color is produced.

**C:** Undiluted Oral Rinse used as the test solution meets the requirements for *Identification test B* under *Calcium Gluconate*, except that a *Standard solution* containing about 0.6 mg of USP Potassium Gluconate RS per mL is used and 15  $\mu$ L of the test solution and the *Standard solution* are applied to the thin-layer chromatographic plate.

**pH** (791): between 5.0 and 7.0.

#### Limit of *p*-chloroaniline—

*Solution A*, *Solution B*, *Mobile phase*, *Diluent*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Chlorhexidine Gluconate Solution*.

*Standard solutions*—Prepare as directed for *Standard solutions* in the test for *Limit of p-chloroaniline* under *Chlorhexidine Gluconate Solution*.

*Test solution*—Transfer 10.0 mL of Oral Rinse to a 25-mL volumetric flask, dilute with *Diluent* to volume, and mix.

*Procedure*—Proceed as directed in the test for *Limit of p-chloroaniline* under *Chlorhexidine Gluconate Solution*. Calculate

the quantity, in  $\mu$ g per mL, of *p*-chloroaniline in the Oral Rinse taken by the formula:

$$2.5C.$$

The limit is 3.0  $\mu$ g per mL.

#### Content of alcohol—

*Internal standard solution*—Dilute 25 mL of *n*-propyl alcohol with water to 500 mL.

*Standard solution*—Transfer about 0.25 g of dehydrated alcohol, accurately weighed, to a 28-mL screw capped vial containing about 3 mL of water. Add 5.0 mL of *Internal standard solution*, and dilute with water to almost fill the vial. Cap the vial, and using a vortex mixer, mix for 15 seconds.

*Test solution*—Transfer about 2.5 g of Oral Rinse, accurately weighed, to a 28-mL screw-capped vial. Add 5.0 mL of *Internal standard solution*, and dilute with water to almost fill the vial. Cap the vial, and using a vortex mixer, mix for 15 seconds.

*Chromatographic system* (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm  $\times$  30-m column, the internal wall of which is coated with a 1.5- $\mu$ m film of liquid phase G27. The column is maintained at about 150° between periods of use. The injection port is equipped with a split injection port with a split ratio of 10:1. The injection port and the detector block temperatures are maintained at about 250° and 275°, respectively. At the time of use the initial column temperature is maintained at about 35° until the alcohol peaks elute, then is increased at a rate of 30° per minute to a final temperature of about 225°. The carrier gas is helium. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are 1.0 for alcohol and about 1.5 for *n*-propyl alcohol; the resolution, *R*, between alcohol and *n*-propyl alcohol is not less than 2; the tailing factor for the alcohol peak is not more than 3.0; and the relative standard deviation for replicate injections is not more than 2%.

*Procedure*—Separately inject equal volumes (about 0.5  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of alcohol ( $C_2H_5OH$ ) in the Oral Rinse taken by the formula:

$$(W_s / W_u)(R_u / R_s)$$

in which *W<sub>s</sub>* is the weight, in g, of dehydrated alcohol taken to prepare the *Standard solution*; *W<sub>u</sub>* is the weight, in g, of Oral Rinse taken to prepare the *Test solution*; and *R<sub>u</sub>* and *R<sub>s</sub>* are the peak response ratios of alcohol to *n*-propyl alcohol obtained from the *Test solution* and the *Standard solution*, respectively: between 90.0% and 115.0% of the labeled amount of alcohol ( $C_2H_5OH$ ) is found.

#### Assay—

*Diluent*, *Solution A*, *Solution B*, *Mobile phase*, *System suitability solution*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Chlorhexidine Gluconate Solution*.

*Assay preparation*—Transfer 5.0 mL of Oral Rinse to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix.

*Procedure*—Proceed as directed in the *Assay* under *Chlorhexidine Gluconate Solution*. Calculate the percentage (w/v) of chlorhexidine gluconate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ ) in the portion of Oral Rinse taken by the formula:

$$(897.76/625.55)(C/500)(r_u / r_s)$$

in which the terms are as defined therein.



## Chlorhexidine Gluconate Solution

$C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$  897.76  
2,4,11,13-Tetraazatetradecanediimidamide, *N,N'*-bis(4-chlorophenyl)-3,12-diimino-, di-D-gluconate;  
1,1'-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] di-D-gluconate [18472-51-0].

### DEFINITION

Chlorhexidine Gluconate Solution is an aqueous solution of chlorhexidine gluconate. It contains NLT 19.0% and NMT 21.0% of chlorhexidine gluconate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ ) (w/v).

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197K)

**Standard solution:** 5 mg/mL of USP Chlorhexidine RS in 70% alcohol. Recrystallize this solution, and dry the crystals at 105° for 1 h.

**Sample solution:** To 1 mL of Chlorhexidine Gluconate Solution add 40 mL of water, and cool in ice. Add 10 N sodium hydroxide, dropwise with stirring, until the solution produces a red color on thiazol yellow paper, and add 1 mL in excess. Filter, wash the precipitate with water until the washings are free from alkali, recrystallize the residue from 70% alcohol, and dry the crystals at 105° for 1 h.

**Acceptance criteria:** Meets the requirements

#### B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Standard solution:** 20 mg/mL of USP Potassium Gluconate RS

**Sample solution:** Dilute 10 mL of Chlorhexidine Gluconate Solution with water to 50 mL. This solution contains 40 mg/mL of chlorhexidine gluconate.

#### Chromatographic system

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 5 µL

**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (5:1:1:3)

**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Develop the chromatogram in a solvent system until the solvent front has moved 10 cm from the point of spotting. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for 10 min.

**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and *R<sub>f</sub>* value to that of the *Standard solution*.

### ASSAY

#### PROCEDURE

**Solution A:** Dissolve 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Mix the resulting solution with acetonitrile (70:30).

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0

Table 1 (Continued)

Time (min)	Solution A (%)	Solution B (%)
10	45	55
15	45	55
16	100	0
21	100	0

**System suitability solution:** 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

**Standard solution:** 50 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*

**Sample stock solution:** Transfer 5.0 mL of Chlorhexidine Gluconate Solution to a 250-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 5.0 mL of the *Sample stock solution* to a 250-mL volumetric flask, and dilute with *Solution A*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm × 25-cm; base-deactivated 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 50 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between chlorhexidine and *p*-chloroaniline

**Relative standard deviation:** NMT 0.73% for chlorhexidine and NMT 5.0% for *p*-chloroaniline

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage (w/v) of chlorhexidine gluconate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ ) in the portion of Chlorhexidine Gluconate Solution taken:

$$\text{Result} = (r_U/r_S) \times (0.25 \times C_S) \times (M_{r1}/M_{r2})$$

*r<sub>U</sub>* = peak response of chlorhexidine from the *Sample solution*

*r<sub>S</sub>* = peak response of chlorhexidine from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)

*M<sub>r1</sub>* = molecular weight of chlorhexidine gluconate, 897.76

*M<sub>r2</sub>* = molecular weight of chlorhexidine acetate, 625.55

**Acceptance criteria:** 19.0%–21.0% (w/v)

### IMPURITIES

#### ORGANIC IMPURITIES

Store the *System suitability solution*, the *Sample solution*, and the *Diluted sample solution* at a temperature of NMT 12°.

**Solution A:** 0.1% (v/v) Trifluoroacetic acid in acetonitrile

**Solution B:** 0.1% (v/v) Trifluoroacetic acid in water

**Solution C:** *Solution A* and *Solution B* (20:80)

**Solution D:** *Solution A* and *Solution B* (90:10)

**Mobile phase:** See *Table 2*. Return to original conditions, and equilibrate the system.



Table 2

Time (min)	Solution C (%)	Solution D (%)
0	100	0
2	100	0
32	80	20
37	80	20
47	70	30
54	70	30

**System suitability solution:** 5.0 mg/mL of USP Chlorhexidine System Suitability Mixture RS in *Solution C*

**Sample solution:** Dilute 1.0 mL of Chlorhexidine Gluconate Solution with *Solution C* to 100.0 mL.

**Diluted sample solution:** Dilute 1.0 mL of *Sample solution* with *Solution C* to 100.0 mL.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; base-deactivated 5-μm packing L1

**Temperatures**

**Column:** 30°

**Autosampler:** NMT 12°

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Peak-to-valley ratio:** NLT 2.0 between chlorhexidine urea and chlorhexidine guanidine

**Resolution:** NLT 3.0 between chlorhexidine oxazinone analog and chlorhexidine amine

#### Analysis

**Samples:** *Sample solution* and *Diluted sample solution*  
Calculate the percentage of each impurity in the portion of Chlorhexidine Gluconate Solution taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of chlorhexidine from the *Diluted sample solution*

$D$  = dilution factor used to prepare the *Diluted sample solution*, 0.01

**Acceptance criteria:** See Table 3. The reporting level for impurities is 0.05%.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorhexidine oxazinone analog	0.23	0.2
Specified unidentified impurity 1	0.24	0.2
Chlorhexidine amine	0.25	0.3
Chlorhexidine guanidine	0.35	1.0
Chlorhexidine urea	0.36	0.2
<i>p</i> -Chlorophenyl urea	0.5	0.2
Chlorhexidine nitrile	0.6	0.4
Chlorhexidine dimer	0.85	0.5
<i>o</i> -Chlorhexidine and specified unidentified impurity 2	0.90–0.91	0.4 <sup>a</sup>

<sup>a</sup> If present, *o*-chlorhexidine and specified unidentified impurity 2 may not be completely resolved by the method. These peaks are integrated together to determine conformance.

Table 3 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorhexidine glucityl triazine	0.96	0.4
Chlorhexidine	1.0	—
Oxochlorhexidine	1.4	0.4
Any individual unspecified impurity	—	0.10
Total impurities	—	3.0

<sup>a</sup> If present, *o*-chlorhexidine and specified unidentified impurity 2 may not be completely resolved by the method. These peaks are integrated together to determine conformance.

#### • LIMIT OF *p*-CHLOROANILINE

**Solution A, Solution B, Mobile phase, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Diluent:** 27.6 g of monobasic sodium phosphate in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL.

**Standard solution:** 1.0 μg/mL of USP *p*-Chloroaniline RS in *Diluent*

**Sample stock solution:** Transfer 5.0 mL of Chlorhexidine Gluconate Solution to a 100-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 10.0 mL of *Sample stock solution* to a 250-mL volumetric flask, and dilute with *Diluent* to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the amount, in μg/mL, of *p*-chloroaniline in the portion of Chlorhexidine Gluconate Solution taken:

$$\text{Result} = (r_U/r_S) \times D \times C_S$$

$r_U$  = peak response of *p*-chloroaniline from the *Sample solution*

$r_S$  = peak response of *p*-chloroaniline from the *Standard solution*

$D$  = dilution factor used to prepare the *Sample solution*, 500

$C_S$  = concentration of *p*-chloroaniline in the *Standard solution* (μg/mL)

**Acceptance criteria:** NMT 500 μg/mL

#### SPECIFIC TESTS

• **SPECIFIC GRAVITY** (841): 1.06–1.07

• **PH** (791): 5.5–7.0, when diluted 1 in 20 with water

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Chlorhexidine RS

USP Chlorhexidine Acetate RS

USP Chlorhexidine System Suitability Mixture RS

The mixture contains chlorhexidine and the following impurities (other impurities may also be present):

Chlorhexidine oxazinone analog;  
(5*R*,6*S*)-2-[(4-chlorophenyl)amino]-5-hydroxy-6-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-5,6-dihydro-4*H*-1,3-oxazin-4-one.

C<sub>13</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>6</sub> 330.72

Chlorhexidine amine;

1-(6-Aminohexyl)-5-(4-chlorophenyl)biguanide.

C<sub>14</sub>H<sub>23</sub>ClN<sub>6</sub> 310.83

Chlorhexidine guanidine;

1-[6-(Carbamimidoylamino)hexyl]-5-(4-chlorophenyl)biguanide.

C<sub>15</sub>H<sub>25</sub>ClN<sub>8</sub> 352.87

Chlorhexidine urea;



$N$ -[6-([(4-Chlorophenyl)carbamimidoyl]-  
 carbamimidoyl)amino]hexyl]carbamimidoyl]urea.  
 $C_{16}H_{26}ClN_9O$  395.89  
 $p$ -Chlorophenyl urea;  
 1-(4-Chlorophenyl)urea.  
 $C_7H_7ClN_2O$  170.60  
 Chlorhexidine nitrile;  
 1-(4-Chlorophenyl)-5-[6-[(cyanocarbamimidoyl)amino]-  
 hexyl]biguanide.  
 $C_{16}H_{24}ClN_9$  377.88  
 Chlorhexidine dimer;  
 1,5-Bis[5-(4-chlorophenyl)biguanidylhexyl]biguanide.  
 $C_{30}H_{47}Cl_2N_{15}$  688.70  
 $o$ -Chlorhexidine;  
 1-(2-Chlorophenyl)-5-[6-([(4-chloro-  
 phenyl)carbamimidoyl]carbamimidoyl)-  
 amino]hexyl]biguanide.  
 $C_{22}H_{30}Cl_2N_{10}$  505.45  
 Specified unidentified impurity 2;  
 Chlorhexidine glucityl triazine;  
 1-(4-Chlorophenyl)-5-[6-([(4-chlorophenyl)amino]-6-  
 [(1,5,2R,3R,4R)-1,2,3,4,5-pentahydroxypentyl]-1,3,5-tri-  
 azin-2-yl)amino]hexyl]biguanide.  
 $C_{28}H_{38}Cl_2N_{10}O_5$  665.57  
 Oxochlorhexidine;  
 $N$ -(4-Chlorophenyl)- $N'$ -[6-([(4-chloro-  
 phenyl)carbamimidoyl]carbamimidoyl)amino]-  
 hexyl]carbamimidoyl]urea.  
 $C_{22}H_{29}Cl_2N_9O$  506.43  
 USP  $p$ -Chloroaniline RS  
 USP Potassium Gluconate RS

## Chlorhexidine Gluconate Topical Solution

### DEFINITION

Chlorhexidine Gluconate Topical Solution is prepared from Chlorhexidine Gluconate Solution. It contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorhexidine gluconate ( $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$ ).

### IDENTIFICATION

- A.** The retention time of the major peak for chlorhexidine from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

- B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 10 mg/mL of USP Potassium Gluconate RS

**Sample solution:** Nominally 20 mg/mL of chlorhexidine gluconate from the Topical Solution

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 10  $\mu$ L

**Developing solvent system:** Alcohol, ethyl acetate, ammonium hydroxide, and water (5:1:1:3)

**Spray reagent:** Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask. Add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Develop the chromatogram in a solvent system until the solvent front has moved 10 cm from the point of spotting. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with *Spray reagent*. Heat the plate at 110° for 10 min.

**Acceptance criteria:** The principal spot from the *Sample solution* corresponds in color, size, and  $R_f$  value to that from the *Standard solution*.

### ASSAY

#### PROCEDURE

**Solution A:** Dissolve 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Prepare a mixture of the resulting solution and acetonitrile (70:30).

**Solution B:** Acetonitrile

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

**System suitability solution:** 50  $\mu$ g/mL of USP Chlorhexidine Acetate RS and 1  $\mu$ g/mL of USP  $p$ -Chloroaniline RS in *Solution A*

**Standard solution:** 50  $\mu$ g/mL of USP Chlorhexidine Acetate RS in *Solution A*.

**Sample solution:** Nominally about 80  $\mu$ g/mL of chlorhexidine gluconate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask, and dilute with methanol to volume. Further dilute a 10-mL portion of this solution with *Solution A* to 50 mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection size:** 50  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The approximate relative retention times for chlorhexidine and  $p$ -chloroaniline are about 1.0 and 1.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 3.0 between chlorhexidine and  $p$ -chloroaniline

**Relative standard deviation:** NMT 2.0% for the chlorhexidine peak, NMT 5.0% for the  $p$ -chloroaniline peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{22}H_{30}Cl_2N_{10} \cdot 2C_6H_{12}O_7$  in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak area of chlorhexidine from the *Sample solution*

$r_S$  = peak area of chlorhexidine from the *Standard solution*

$C_S$  = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* ( $\mu$ g/mL)

$C_U$  = nominal concentration of chlorhexidine gluconate in the *Sample solution* ( $\mu$ g/mL)

$M_{r1}$  = molecular weight of chlorhexidine gluconate, 897.76

$M_{r2}$  = molecular weight of chlorhexidine acetate, 625.55



Acceptance criteria: 90.0%–110.0%

## IMPURITIES

### Organic Impurities

#### PROCEDURE: LIMIT OF *p*-CHLOROANILINE

**Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the Assay.

**Standard solution:** 1.0 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

**Sample solution:** Nominally 0.4 mg/mL of chlorhexidine gluconate from the Topical Solution, prepared as follows. Transfer an amount of Topical Solution, equivalent to 40 mg of chlorhexidine gluconate, to a 100-mL volumetric flask, and dilute with *Solution A* to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

#### Acceptance criteria

The *p*-chloroaniline peak area from the *Sample solution* is NMT the *p*-chloroaniline peak area from the *Standard solution* (equivalent to NMT 500 ppm in the portion of Chlorhexidine Gluconate Solution used to prepare the Topical Solution).

## SPECIFIC TESTS

#### PH (791): 5.0–7.0

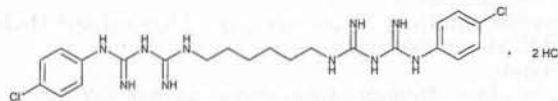
## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.

#### USP REFERENCE STANDARDS (11)

USP Chlorhexidine Acetate RS  
USP *p*-Chloroaniline RS  
USP Potassium Gluconate RS

## Chlorhexidine Hydrochloride



$C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$  578.37  
2,4,11,13-Tetraazatetradecanediimidamide, *N,N'*-bis(4-chlorophenyl)-3,12-diimino-, dihydrochloride; 1,1'-Hexamethylenebis[5-(*p*-chlorophenyl)biguanide] dihydrochloride [3697-42-5].

## DEFINITION

Chlorhexidine Hydrochloride contains NLT 98.0% and NMT 102.0% of chlorhexidine hydrochloride ( $C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$ ), calculated on the dried basis.

## IDENTIFICATION

#### A. INFRARED ABSORPTION (197K)

**Sample:** Dissolve 0.3 g in 10 mL of 6 N hydrochloric acid. Add 40 mL of water, filter if necessary, and cool the solution in ice. Add 10 N sodium hydroxide, dropwise with stirring, until the solution is alkaline to thiazol yellow paper, and add 1 mL in excess. Filter, wash the precipitate with water until the washings are free from alkali, recrystallize the residue from 70% alcohol, and dry the crystals at 105° for 1 h.

**Standard:** 5 mg/mL of USP Chlorhexidine RS in 70% alcohol. Recrystallize this solution, and dry the crystals at 105° for 1 h.

**Acceptance criteria:** Meets the requirements

• **B. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

## ASSAY

### PROCEDURE

**Solution A:** 27.6 g of monobasic sodium phosphate and 10 mL of triethylamine in 1.5 L of water. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to 2000 mL. Prepare a mixture of acetonitrile and this solution (3:7).

**Solution B:** Acetonitrile

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
9	100	0
10	45	55
15	45	55
16	100	0
21	100	0

**System suitability solution:** 50 µg/mL of USP Chlorhexidine Acetate RS and 1 µg/mL of USP *p*-Chloroaniline RS in *Solution A*

**Standard solution:** 50 µg/mL of USP Chlorhexidine Acetate RS in *Solution A*

**Sample solution:** 50 µg/mL of Chlorhexidine Hydrochloride in *Solution A*

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm × 25-cm; base-deactivated 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 50 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for chlorhexidine and *p*-chloroaniline are about 1.0 and 1.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 3 between chlorhexidine and *p*-chloroaniline

**Relative standard deviation:** NMT 0.73% for chlorhexidine and NMT 5.0% for *p*-chloroaniline

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of chlorhexidine hydrochloride ( $C_{22}H_{30}Cl_2N_{10} \cdot 2HCl$ ) in the portion of Chlorhexidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of chlorhexidine from the *Sample solution*

$r_S$  = peak response of chlorhexidine from the *Standard solution*

$C_S$  = concentration of USP Chlorhexidine Acetate RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Chlorhexidine Hydrochloride in the *Sample solution* (µg/mL)

$M_{r1}$  = molecular weight of chlorhexidine hydrochloride, 578.37

$M_{r2}$  = molecular weight of chlorhexidine acetate, 625.55



Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

- **ORGANIC IMPURITIES**

Store the *System suitability solution*, the *Sample solution*, and the *Diluted sample solution* at a temperature of NMT 12°.

**Solution A:** 0.1% (v/v) Trifluoroacetic acid in acetonitrile

**Solution B:** 0.1% (v/v) Trifluoroacetic acid in water

**Solution C:** *Solution A* and *Solution B* (20:80)

**Solution D:** *Solution A* and *Solution B* (90:10)

**Mobile phase:** See *Table 2*. Return to original conditions, and equilibrate the system.

**Table 2**

Time (min)	Solution C (%)	Solution D (%)
0	100	0
2	100	0
32	80	20
37	80	20
47	70	30
54	70	30

**System suitability solution:** 5.0 mg/mL of USP Chlorhexidine System Suitability Mixture RS in *Solution C*. See *Table 3* for the relative retention times of the main components of the mixture.

**Table 3**

Components of USP Chlorhexidine System Suitability Mixture RS	Relative Retention Time
Chlorhexidine oxazinone analog	0.23
Chlorhexidine amine	0.25
Chlorhexidine guanidine	0.35
Chlorhexidine urea	0.36
<i>p</i> -Chlorophenyl urea	0.5
Chlorhexidine nitrile	0.6
Chlorhexidine dimer	0.85
<i>o</i> -Chlorhexidine	0.90
Specified unidentified impurity 2	0.91
Chlorhexidine glucityl triazine	0.96
Chlorhexidine	1.0
Oxochlorhexidine	1.4

**Sample solution:** 1.3 mg/mL of Chlorhexidine Hydrochloride in *Solution C*

**Diluted sample solution:** Dilute 1.0 mL of *Sample solution* with *Solution C* to 100.0 mL.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; base-deactivated 5-μm packing L1

**Temperatures**

**Column:** 30°

**Autosampler:** NMT 12°

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 μL

### System suitability

**Sample:** *System suitability solution*

**Suitability requirements**

**Peak-to-valley ratio:** NLT 2.0 between chlorhexidine urea and chlorhexidine guanidine

### Analysis

**Samples:** *Sample solution* and *Diluted sample solution*  
Calculate the percentage of each impurity in the portion of Chlorhexidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of chlorhexidine from the *Diluted sample solution*

$D$  = dilution factor used to prepare the *Diluted sample solution*, 0.01

**Acceptance criteria:** See *Table 4*. The reporting level for impurities is 0.05%.

**Table 4**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Chlorhexidine guanidine	0.35	0.15
Chlorhexidine nitrile	0.6	0.15
Chlorhexidine dimer	0.85	0.2
<i>o</i> -Chlorhexidine and specified unidentified impurity 2	0.90–0.91	0.4 <sup>a</sup>
Chlorhexidine	1.0	—
Oxochlorhexidine	1.4	0.4
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

<sup>a</sup> If present, *o*-chlorhexidine and specified unidentified impurity 2 may not be completely resolved by the method. These peaks are integrated together to determine conformance.

- **LIMIT OF *p*-CHLOROANILINE**

**Solution A, Solution B, Mobile phase, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard solution:** 1.0 μg/mL of USP *p*-Chloroaniline RS in *Solution A*

**Sample solution:** 2.0 mg/mL of Chlorhexidine Hydrochloride in *Solution A*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the amount, in ppm, of *p*-chloroaniline in the portion of Chlorhexidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 0.001 \times 10^6$$

$r_U$  = peak response of *p*-chloroaniline from the *Sample solution*

$r_S$  = peak response of *p*-chloroaniline from the *Standard solution*

$C_S$  = concentration of *p*-chloroaniline in the *Standard solution* (μg/mL)

$C_U$  = concentration of Chlorhexidine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 500 ppm

### SPECIFIC TESTS

- **LOSS ON DRYING** (731)

**Analysis:** Dry at 105° to constant weight.

**Acceptance criteria:** NMT 1.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

- **USP REFERENCE STANDARDS** (11)

USP Chlorhexidine RS

USP Chlorhexidine Acetate RS

USP Chlorhexidine System Suitability Mixture RS

The mixture contains chlorhexidine and the following impurities (other impurities may also be present):



Chlorhexidine oxazinone analog;  
(5*R*,6*S*)-2-[(4-Chlorophenyl)amino]-5-hydroxy-6-[(1*R*,2*R*)-1,2,3-trihydroxypropyl]-5,6-dihydro-4*H*-1,3-oxazin-4-one.

$C_{13}H_{15}ClN_2O_6$  330.72

Chlorhexidine amine;

1-(6-Aminoethyl)-5-(4-chlorophenyl)biguanide.

$C_{14}H_{23}ClN_6$  310.83

Chlorhexidine guanidine;

1-[6-(Carbamimidoylamino)hexyl]-5-(4-chlorophenyl)biguanide.

$C_{15}H_{25}ClN_8$  352.87

Chlorhexidine urea;

*N*-[6-[[[(4-Chlorophenyl)carbamimidoyl]-carbamimidoyl]amino]hexyl]carbamimidoyl]urea.

$C_{16}H_{26}ClN_9O$  395.89

*p*-Chlorophenyl urea;

1-(4-Chlorophenyl)urea.

$C_7H_7ClN_2O$  170.60

Chlorhexidine nitrile;

1-(4-Chlorophenyl)-5-[6-[(cyanocarbamidoyl)amino]hexyl]biguanide.

$C_{16}H_{24}ClN_9$  377.88

Chlorhexidine dimer;

1,5-Bis[5-(4-chlorophenyl)biguanidylhexyl]biguanide.

$C_{30}H_{47}Cl_2N_{15}$  688.70

*o*-Chlorhexidine;

1-(2-Chlorophenyl)-5-[6-[(4-chlorophenyl)carbamimidoyl]carbamimidoyl]-amino]hexyl]biguanide.

$C_{22}H_{30}Cl_2N_{10}$  505.45

Specified unidentified impurity 2;

Chlorhexidine glucityl triazine;

1-(4-Chlorophenyl)-5-[6-[(4-[(4-chlorophenyl)amino]-6-[(1*S*,2*R*,3*R*,4*R*)-1,2,3,4,5-pentahydroxypentyl]-1,3,5-triazin-2-yl)amino]hexyl]biguanide.

$C_{28}H_{38}Cl_2N_{10}O_5$  665.57

Oxochlorhexidine;

*N*-(4-Chlorophenyl)-*N'*-[6-[(4-chlorophenyl)carbamimidoyl]carbamimidoyl]-amino]hexyl]carbamimidoyl]urea.

$C_{22}H_{29}Cl_2N_9O$  506.43

USP *p*-Chloroaniline RS

100-mL volumetric flasks, and dilute the contents of each flask with water to volume. These *Standard solutions* contain 0.5, 1.0, 1.5, and 2.0 µg/mL of copper, respectively.

**Sample solution:** Transfer 100 mg of previously dried Chlorophyllin Copper Complex Sodium to a Kjeldahl flask. Add 2.0 mL of sulfuric acid, 1.0 mL of nitric acid, and 1.0 mL of hydrogen peroxide, and carefully heat under a fume hood until a light green color is obtained. [NOTE—If the solution has any hint of a brown tint, continue to add 0.5-mL portions of nitric acid until a green color is obtained.] Cool, transfer the contents quantitatively to a 1000-mL volumetric flask with several portions of water, dilute the contents of the flask with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, and dilute with water to volume.

#### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometry

**Lamp:** Copper hollow-cathode

**Flame:** Air-acetylene

**Analytical wavelength:** Copper emission line of 324.8 nm

**Blank:** Water

#### Analysis

**Samples:** *Standard solutions* and *Sample solution*

Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus the concentration, in µg/mL, of copper, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, *C*, in µg/mL, of copper in the *Sample solution*.

Calculate the percentage of copper in the portion of Chlorophyllin Copper Complex Sodium taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

*C* = concentration of the *Sample solution* determined from the graph (µg/mL)

*W* = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (mg)

*V* = final volume of *Sample solution*, 5000 mL

*F* = conversion factor, 1000 µg/mg

**Acceptance criteria:** NLT 4.25% on the dried basis

#### • CONTENT OF CHELATED COPPER

**Analysis:** Calculate the percentage of chelated copper in the portion of Chlorophyllin Copper Complex Sodium taken by subtracting the percentage of ionic copper found in the test for *Limit of Ionic Copper* from the percentage of total copper found in the test for *Content of Total Copper*.

**Acceptance criteria:** NLT 4.0% on the dried basis

#### • CONTENT OF SODIUM

**Standard stock solution:** 100 µg/mL of sodium. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer to a 1000-mL volumetric flask, and dilute with water to volume.

**Standard solutions:** Transfer to each of four 100-mL volumetric flasks 10 mL of a nonionic wetting agent solution (1 in 500). To each flask add, respectively, 2.5, 5.0, 10.0, and 15.0 mL of the *Standard stock solution*, and dilute with water to volume. These *Standard solutions* contain 2.5, 5.0, 10.0, and 15.0 µg/mL of sodium, respectively.

**Blank:** Transfer 10 mL of a nonionic wetting agent solution (1 in 500) into a 100-mL volumetric flask, and dilute with water to volume.

**Sample solution:** Transfer 100 mg of Chlorophyllin Copper Complex Sodium to a 1000-mL volumetric flask. Add 100 mL of a solution of nonionic wetting agent (1 in 500) and 400 mL of water, and shake by

## Chlorophyllin Copper Complex Sodium

### DEFINITION

Chlorophyllin Copper Complex Sodium contains sodium salts of copper-chelated chlorophyll derivatives. It contains no artificial coloring.

### IDENTIFICATION

#### • A. ULTRAVIOLET-VISIBLE SPECTROSCOPY (857) (in the visible region)

**Sample solution:** 10 µg/mL

**Medium:** pH 7.5 phosphate buffer, prepared by mixing 0.15 M dibasic sodium phosphate and 0.15 M monobasic potassium phosphate (21:4)

**Acceptance criteria:** The ratio of  $A_{405}/A_{630}$  is 3.0–3.9.

### OTHER COMPONENTS

#### • CONTENT OF TOTAL COPPER

**Stock solution 1:** 1000 µg/mL of copper. Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, and dilute with 0.2 N nitric acid to volume. [NOTE—Store in a polyethylene bottle.]

**Stock solution 2:** 10 µg/mL of copper. Transfer 5.0 mL of *Stock solution 1* into a 500-mL volumetric flask, and dilute with water to volume.

**Standard solutions:** Transfer 5.0, 10.0, 15.0, and 20.0 mL, respectively, of *Stock solution 2* to separate



mechanical means for 5 min. Dilute with water to volume.

#### Analysis

**Samples:** *Standard solutions* and *Sample solution*

Set the flame photometer for maximum transmission at a wavelength of 589 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage of transmittance of the other *Standard solutions*, and plot the percentage of transmittance versus the concentration, in  $\mu\text{g/mL}$ , of sodium. Read the percentage of transmittance of the *Sample solution*, and from the graph read the concentration,  $C$ , in  $\mu\text{g/mL}$ , of sodium in the *Sample solution*.

Calculate the percentage of sodium in the portion of Chlorophyllin Copper Complex Sodium taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

- $C$  = concentration of the *Sample solution* determined from the graph ( $\mu\text{g/mL}$ )  
 $W$  = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (mg)  
 $V$  = volume of *Sample solution*, 1000 mL  
 $F$  = conversion factor, 1000  $\mu\text{g/mg}$

**Acceptance criteria:** 5%–7% on the dried basis

- **NITROGEN DETERMINATION**, *Method I* (461): NLT 4.0%

#### IMPURITIES

##### • LIMIT OF IONIC COPPER

**Standard solutions:** Prepare as directed in the test for *Content of Total Copper*.

**Sample solution:** Transfer 100 mg of Chlorophyllin Copper Complex Sodium to a 150-mL conical flask. Add 75 mL of water, and shake by mechanical means for 3 min. Adjust with 1 N hydrochloric acid to a pH of 3.0, transfer the suspension thus obtained to a 100-mL volumetric flask, and dilute with water to volume. Filter this suspension, discarding the first 10 mL of the filtrate. Use the clear filtrate for analysis.

#### Analysis

**Samples:** *Standard solutions* and *Sample solution*  
 Proceed as directed in the test for *Content of Total Copper*.

Calculate the percentage of ionic copper in the portion of Chlorophyllin Copper Complex Sodium taken:

$$\text{Result} = (C/W) \times (V/F) \times 100$$

- $C$  = concentration of the *Sample solution* determined from the graph ( $\mu\text{g/mL}$ )  
 $W$  = weight of Chlorophyllin Copper Complex Sodium taken to prepare the *Sample solution* (in mg on the dried basis)  
 $V$  = volume of *Sample solution*, 100 mL  
 $F$  = conversion factor, 1000  $\mu\text{g/mg}$
- Acceptance criteria:** NMT 0.25% on the dried basis
- **RESIDUE ON IGNITION** (281): NMT 30% on the dried basis
  - **ARSENIC**, *Method II* (211): NMT 3 ppm
  - **LEAD** (251): NMT 10 ppm
  - **IRON** (241): NMT 0.50%

#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for absence of *Escherichia coli* and *Salmonella* species.
- **pH** (791): 9.5–10.7, in a solution (1 in 100)
- **LOSS ON DRYING** (731): Dry a sample at 105° for 2 h: it loses NMT 5% of its weight.
- **TEST FOR FLUORESCENCE**  
**Sample solution:** 10 mg/mL  
**Analysis:** Apply 10  $\mu\text{L}$  of *Sample solution* on filter paper, allow to dry, and examine the area of application

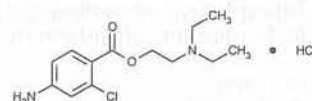
under long-wavelength UV light through a red optical filter.

**Acceptance criteria:** No fluorescence is visible.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

## Chloroprocaine Hydrochloride



$\text{C}_{13}\text{H}_{19}\text{ClN}_2\text{O}_2 \cdot \text{HCl}$  307.22

Benzoic acid, 4-amino-2-chloro-, 2-(diethylamino)ethyl ester, monohydrochloride.

2-(Diethylamino)ethyl 4-amino-2-chlorobenzoate monohydrochloride [3858-89-7].

» Chloroprocaine Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of  $\text{C}_{13}\text{H}_{19}\text{ClN}_2\text{O}_2 \cdot \text{HCl}$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Chloroprocaine Hydrochloride RS

#### Identification—

**A:** *Infrared Absorption* (197K).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10  $\mu\text{g}$  per mL.

*Medium:* pH 4.5 buffer solution [prepared by dissolving 13.61 g of monobasic potassium phosphate in 750 mL of water, adjusting to a pH of  $4.5 \pm 0.1$  with potassium hydroxide solution (1 in 180) and diluting with water to 1000 mL]. Absorptivities at 290 nm, calculated on the dried basis, do not differ by more than 3.0%.

**C:** It meets the requirements of the tests for *Chloride* (191).

**Melting range**, *Class I* (741): between 173° and 176°.

**Acidity**—Dissolve 1.0 g in 25 mL of water, add 2 drops of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 1.8 mL is required to produce a yellow color.

**Loss on drying** (731)—Dry about 500 mg, accurately weighed, at 105° for 2 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

#### Related compounds—

**4-Amino-2-chlorobenzoic acid**—Using the chromatograms obtained as directed for the *Assay*, calculate the percentage of 4-amino-2-chlorobenzoic acid ( $\text{C}_7\text{H}_6\text{ClNO}_2$ ) in the Chloroprocaine Hydrochloride taken by the formula:

$$10,000(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of 4-amino-2-chlorobenzoic acid in the *Standard preparation*;  $W$  is the quantity, in mg, of Chloroprocaine Hydrochloride taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the 4-amino-2-chlorobenzoic acid peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 0.625% is found.



**Assay—**

**Mobile phase**—Dissolve 800 mg of sodium 1-heptanesulfonate in 740 mL of water, add 200 mL of acetonitrile, 50 mL of methanol, and 10 mL of glacial acetic acid, and mix. Filter and degas this solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard 4-amino-2-chlorobenzoic acid solution**—Dissolve an accurately weighed quantity of recrystallized 4-amino-2-chlorobenzoic acid in methanol to obtain a solution having a known concentration of about 0.2 mg per mL.

**Standard preparation**—Transfer about 50 mg of USP Chloroprocaine Hydrochloride RS, accurately weighed, to a 50-mL volumetric flask containing 5.0 mL of *Standard 4-amino-2-chlorobenzoic acid solution*, add 15 mL of methanol, swirl to dissolve, dilute with water to volume, and mix. This solution contains about 1 mg of USP Chloroprocaine Hydrochloride RS and 0.02 mg of 4-amino-2-chlorobenzoic acid per mL.

**System suitability solution**—Mix equal volumes of *Standard 4-amino-2-chlorobenzoic acid solution* and *Standard preparation*.

**Assay preparation**—Transfer about 100 mg of Chloroprocaine Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in 40 mL of methanol, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 278-nm detector and a 3.9-mm × 30-cm column containing packing L1. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the responses as directed for *Procedure*: the relative retention times are about 0.35 for 4-amino-2-chlorobenzoic acid and 1.0 for chloroprocaine; and the resolution,  $R$ , between 4-amino-2-chlorobenzoic acid and chloroprocaine is not less than 5.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative standard deviation determined from chloroprocaine obtained from replicate injections is not more than 1.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for 4-amino-2-chlorobenzoic acid and chloroprocaine. Calculate the quantity, in mg, of  $C_{13}H_{19}ClN_2O_2 \cdot HCl$  in the portion of Chloroprocaine Hydrochloride taken by the formula:

$$100C(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Chloroprocaine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the chloroprocaine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chloroprocaine Hydrochloride Injection

» Chloroprocaine Hydrochloride Injection is a sterile solution of Chloroprocaine Hydrochloride in Water for Injection. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of chloroprocaine hydrochloride ( $C_{13}H_{19}ClN_2O_2 \cdot HCl$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards (11)—**

USP Chloroprocaine Hydrochloride RS

**Identification**—Dissolve 60 mg of USP Chloroprocaine Hydrochloride RS in 10 mL of water in a 60-mL separator, and in a second 60-mL separator mix a volume of Injection, equivalent to 60 mg of chloroprocaine hydrochloride, with sufficient water to obtain 10 mL of solution. Add 5 mL of dilute ammonium hydroxide (4 in 10) to each, mix, and immediately extract each with four 10-mL portions of chloroform, passing the extracts from the Reference Standard and the test specimen through cotton filters into separate 50-mL volumetric flasks. Dilute each with chloroform to volume, and mix. Add a mixture of chloroform and methanol (4:1) to a suitable chromatographic chamber arranged for thin-layer chromatography (see *Chromatography* (621)), cover the chamber, and allow the system to equilibrate for 15 minutes. Apply separately 10- $\mu$ L portions of the chloroform solutions obtained from the Reference Standard and the test specimen to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution ( $R_f$  about 0.40). After viewing, spray the chromatogram with iodoplatinate TS: violet-blue colored spots, characteristic of tertiary nitrogen compounds, are visible.

**pH** (791): between 2.7 and 4.0.

**Related compounds—**

**4-Amino-2-chlorobenzoic acid**—Using the chromatograms obtained as directed for the *Assay*, calculate the percentage of 4-amino-2-chlorobenzoic acid ( $C_7H_6ClNO_2$ ) in the chloroprocaine hydrochloride contained in the Injection taken by the formula:

$$10,000(C / W_o)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of 4-amino-2-chlorobenzoic acid in the *Standard preparation*;  $W_o$  is the quantity, in mg, of chloroprocaine hydrochloride in the portion of Injection taken to prepare the *Assay preparation*, determined as directed in the *Assay preparation*; and  $r_U$  and  $r_S$  are the 4-amino-2-chlorobenzoic acid peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 3.0% is found.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay—**

**Mobile phase**, *Standard 4-amino-2-chlorobenzoic acid solution*, *Standard preparation*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the *Assay under Chloroprocaine Hydrochloride*.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of chloroprocaine hydrochloride, to a 100-mL volumetric flask, add 40 mL of methanol, dilute with water to volume, and mix.

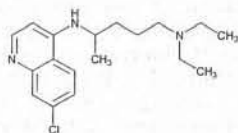
**Procedure**—Proceed as directed in the *Assay under Chloroprocaine Hydrochloride*. Calculate the quantity, in mg, of chloroprocaine hydrochloride ( $C_{13}H_{19}ClN_2O_2 \cdot HCl$ ) in each mL of the Injection taken by the formula:

$$100(C / V)(r_U / r_S)$$

in which  $V$  is the volume, in mL, of Injection taken, and the other terms are as defined therein.



## Chloroquine



$C_{18}H_{26}ClN_3$  319.87  
 1,4-Pentanediamine, *N*-(7-chloro-4-quinoliny)-*N*′, *N*′-diethyl-;  
 7-Chloro-4-[[4-(diethylamino)-1-methylbutyl]amino]quinoline [54-05-7].

### DEFINITION

Chloroquine contains NLT 98.0% and NMT 102.0% of chloroquine ( $C_{18}H_{26}ClN_3$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181)**  
**Standard solution:** Proceed as directed in the chapter except use chloroform instead of carbon disulfide in the test.  
**Sample solution:** 8.75 mg/mL of chloroquine prepared as follows. Dissolve 35 mg of Chloroquine in 4 mL of chloroform. Pass through a dry filter.  
**Analysis:** Determine the absorption of the *Standard solution* and *Sample solution* without delay in 1-mm cells between 7 and 15  $\mu$ m, using chloroform in a matched cell as a blank.  
**Acceptance criteria:** The IR absorption spectrum of the *Sample solution* so obtained exhibits maxima only at the same wavelengths as that of the *Standard solution*.
- **B. ULTRAVIOLET ABSORPTION (197U)**  
**Medium:** Dilute hydrochloric acid (1 in 1000)  
**Sample solution:** 10  $\mu$ g/mL  
**Acceptance criteria:** The ratio  $A_{343}/A_{329}$  is 1.00–1.15.

### ASSAY

- **PROCEDURE**  
**Sample solution:** Dissolve 250 mg of Chloroquine in 50 mL of glacial acetic acid. Add crystal violet TS.  
**Analysis:** Titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 15.99 mg of chloroquine ( $C_{18}H_{26}ClN_3$ ).  
**Acceptance criteria:** 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.2%

### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE (741):** 87°–92°
- **LOSS ON DRYING (731)**  
**Analysis:** Dry a sample at 105° for 2 h.  
**Acceptance criteria:** NMT 2.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.
- **USP REFERENCE STANDARDS (11)**  
 USP Chloroquine Phosphate RS

## Chloroquine Hydrochloride Injection

$C_{18}H_{26}ClN_3 \cdot 2HCl$  392.79  
 1,4-Pentanediamine, *N*-(7-chloro-4-quinoliny)-*N*′, *N*′-diethyl-, dihydrochloride;

7-(Chloro-4-[[4-diethylamino)-1-methylbutyl]amino]quinoline dihydrochloride [3545-67-3].

### DEFINITION

Chloroquine Hydrochloride Injection is a sterile solution of Chloroquine in Water for Injection prepared with the aid of Hydrochloric Acid. It contains NLT 47.5 mg and NMT 52.5 mg of chloroquine hydrochloride ( $C_{18}H_{26}ClN_3 \cdot 2HCl$ ) in each mL.

### IDENTIFICATION

#### • A. ULTRAVIOLET ABSORPTION

**Sample solution:** Prepare as directed in the Assay.  
**Standard solution:** 7.5  $\mu$ g/mL of USP Chloroquine Phosphate RS prepared similarly to the *Sample solution*  
**Instrumental conditions**  
**Mode:** UV  
**Wavelength range:** 329–343 nm  
**Analysis**  
**Samples:** *Sample solution* and *Standard solution*  
 Calculate the absorbance ratio:

$$\text{Result} = A_{343}/A_{329}$$

$A_{343}$  = absorbance of the *Sample solution* at 343 nm  
 $A_{329}$  = absorbance of the *Sample solution* at 329 nm  
**Acceptance criteria:** The peak maxima and minima of the *Sample solution* correspond to those of the *Standard solution*; the absorbance ratio is 1.00–1.15.

#### • B.

**Sample solution:** 20 mL of a 1-mg/mL solution of chloroquine hydrochloride from Injection, diluted with water

**Analysis 1:** To the *Sample solution* add 5 mL of trinitrophenol TS.

**Acceptance criteria 1:** A yellow precipitate is formed.

**Analysis 2:** Filter, wash the precipitate with water until the last washing is colorless, and dry over silica gel.

[CAUTION—Picrates may explode.]

**Acceptance criteria 2:** The precipitate melts at 205°–210°.

- **C. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

### ASSAY

#### • PROCEDURE

**Diluent 1:** Dilute hydrochloric acid (1 in 100)

**Diluent 2:** Dilute hydrochloric acid (1 in 1000)

**Standard solution:** 10  $\mu$ g/mL of USP Chloroquine Phosphate RS in *Diluent 2*

**Sample stock solution:** 150  $\mu$ g/mL of chloroquine hydrochloride prepared as follows. Transfer a volume of Injection, nominally 150 mg of chloroquine hydrochloride, to a 1-L volumetric flask, and dilute with water to volume.

**Sample solution:** 7.5  $\mu$ g/mL of chloroquine hydrochloride prepared as follows. Transfer 5.0 mL of the *Sample stock solution* to a 100.0-mL volumetric flask, add 10.0 mL of *Diluent 1*, and dilute with water to volume.

#### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** Maxima at about 343 nm

**Blank:** Water

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the concentration, in mg/mL, of chloroquine hydrochloride ( $C_{18}H_{26}ClN_3 \cdot 2HCl$ ) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times (V/V_i) \times (M_{r1}/M_{r2})$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Chloroquine Phosphate RS in the *Standard solution* (mg/mL)



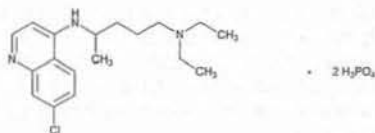
- $D$  = dilution factor for the preparation of the *Sample solution*, 20  
 $V$  = volume of the *Sample stock solution*, 1000 mL  
 $V_i$  = volume of Injection taken for the *Sample stock solution* (mL)  
 $M_{r1}$  = molecular weight of chloroquine hydrochloride, 392.79  
 $M_{r2}$  = molecular weight of chloroquine phosphate, 515.86  
 Acceptance criteria: 47.5–52.5 mg/mL

**SPECIFIC TESTS**

- **pH (791):** 5.5–6.5
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.7 USP Endotoxin Unit/mg of chloroquine hydrochloride.
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products (1)*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS (11)**  
 USP Chloroquine Phosphate RS  
 USP Endotoxin RS

**Chloroquine Phosphate**

$C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$  515.86  
 1,4-Pentanediamine,  $N^4$ -(7-chloro-4-quinolyl)- $N^1,N^1$ -diethyl-, phosphate (1:2);  
 7-Chloro-4-[[4-(diethylamino)-1-methylbutyl]amino]quinoline phosphate (1:2) [50-63-5].

**DEFINITION**

Chloroquine Phosphate contains NLT 98.0% and NMT 102.0% of chloroquine phosphate ( $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B. ULTRAVIOLET ABSORPTION (197U)**  
 Medium: Dilute hydrochloric acid (1 in 1000)  
 Sample solution: 10 µg/mL  
 Ratio:  $A_{343}/A_{329}$ , 1.00–1.15
- **C.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE**

**Buffer:** 1.4 g/L of anhydrous dibasic sodium phosphate in water. Adjust with 10% phosphoric acid to a pH of 3.0.

**Mobile phase:** 0.4% triethylamine in methanol and *Buffer* (70:30)

**System suitability solution:** 2.0 µg/mL each of USP Chloroquine Phosphate RS, USP Phenol RS, USP Hydroxychloroquine Sulfate RS, USP Chloroquine Related Compound A RS, USP Chloroquine Related Compound D RS, USP Chloroquine Related Compound E RS, and USP Chloroquine Related Compound G RS in *Mobile phase*

**Standard solution:** 0.3 mg/mL of USP Chloroquine Phosphate RS in *Mobile phase*. Sonicate to dissolve if necessary.

**Sample solution:** 0.3 mg/mL of Chloroquine Phosphate in *Mobile phase*. Sonicate to dissolve if necessary.

**Chromatographic system**

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 260 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for the corresponding relative retention times.]

**Suitability requirements**

**Resolution:** NLT 2.0 between chloroquine and chloroquine related compound A, *System suitability solution*

**Tailing factor:** NMT 2.0 for chloroquine, *Standard solution*

**Relative standard deviation:** NMT 0.7% for chloroquine, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of chloroquine phosphate ( $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ ) in the portion of Chloroquine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Chloroquine Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Chloroquine Phosphate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

**IMPURITIES****ORGANIC IMPURITIES**

**Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** Use the *System suitability solution*.

**Sample solution:** 2 mg/mL of Chloroquine Phosphate in *Mobile phase*

**System suitability**

**Sample:** *System suitability solution*

[NOTE—See *Table 1* for the corresponding relative retention times.]

**Suitability requirements**

**Resolution:** NLT 2.0 between chloroquine and chloroquine related compound A and NLT 2 between adjacent impurities

**Tailing factor:** NMT 2.0 for peaks corresponding to chloroquine phosphate, phenol, hydroxychloroquine sulfate, chloroquine related compound A, chloroquine related compound D, chloroquine related compound E, and chloroquine related compound G

**Relative standard deviation:** NMT 5.0% for chloroquine phosphate, phenol, hydroxychloroquine sulfate, chloroquine related compound A, chloroquine related compound D, chloroquine related compound E, and chloroquine related compound G

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Chloroquine Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*



- $r_s$  = peak response of the corresponding USP Reference Standard from the *Standard solution*
- $C_s$  = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)
- $C_u$  = concentration of Chloroquine Phosphate in the *Sample solution* (mg/mL)

Calculate the percentage of chloroquine related compound G and any other unspecified impurity in the portion of Chloroquine Phosphate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of chloroquine *N*-oxide or any other impurity from the *Sample solution*
- $r_s$  = peak response of USP Chloroquine Phosphate RS from the *Standard solution*
- $C_s$  = concentration of USP Chloroquine Phosphate RS in the *Standard solution* (mg/mL)
- $C_u$  = concentration of Chloroquine Phosphate in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 1. Disregard any peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Phenol	0.2	0.1
Chloroquine related compound G	0.27	0.1
Chloroquine related compound D	0.42	0.50
Hydroxychloroquine sulfate	0.49	0.1
Chloroquine related compound A	0.73	0.1
Chloroquine phosphate	1.0	—
Chloroquine related compound E	1.5	0.1
Any other individual impurity	—	0.10
Total impurities	—	2.0

### SPECIFIC TESTS

#### • LOSS ON DRYING (731)

Analysis: Dry a sample at 105° for 16 h.

Acceptance criteria: NMT 2.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

#### • USP REFERENCE STANDARDS (11)

- USP Chloroquine Phosphate RS
- USP Chloroquine Related Compound A RS  
4,7-Dichloroquinoline.  
 $C_9H_5Cl_2N$  198.05
- USP Chloroquine Related Compound D RS  
Monoethyl chloroquine;  
7-Chloro-4-[[4-(ethylamino)-1-methylbutyl]amino]quinoline.  
 $C_{16}H_{22}ClN_3$  291.82
- USP Chloroquine Related Compound E RS  
5-Chloroquine isomer;  
 $N^4$ -(5-Chloroquinolin-4-yl)- $N^1,N^1$ -diethylpentane-1,4-diamine oxalate.  
 $C_{18}H_{26}ClN_3 \cdot C_2H_2O_4$  409.91
- USP Chloroquine Related Compound G RS  
4-[[7-Chloroquinolin-4-yl]amino]- $N,N$ -diethylpentan-1-amine oxide sulfate.  
 $C_{18}H_{26}Cl_3NO \cdot H_2SO_4$  433.95

USP Hydroxychloroquine Sulfate RS  
USP Phenol RS

## Chloroquine Phosphate Compounded Oral Suspension

### DEFINITION

Chloroquine Phosphate Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of chloroquine phosphate ( $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ ).

Prepare Chloroquine Phosphate Compounded Oral Suspension 15 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Chloroquine Phosphate tablets <sup>a</sup> equivalent to	1.5 g of chloroquine phosphate
Vehicle: a 1:1 mixture of Ora-Sweet <sup>b</sup> and Ora-Plus <sup>b</sup> , a sufficient quantity to make	100 mL

<sup>a</sup> Aralen 500-mg tablets, Sanofi-Winthrop, NY.

<sup>b</sup> Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Chloroquine Phosphate tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a chloroquine phosphate liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.

### ASSAY

#### • PROCEDURE

**Buffer solution:** 20 mM 1-heptanesulfonic acid adjusted to a pH of 3.4

**Mobile phase:** Acetonitrile and *Buffer solution* (34:66). Filter and degas.

**Standard solution:** 150 µg/mL of USP Chloroquine Phosphate RS in *Mobile phase*

**Sample solution:** Shake thoroughly by hand each bottle of Oral Suspension. Pipet 1.0 mL of the Oral Suspension into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 340 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time for chloroquine phosphate is about 9.4 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 2% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chloroquine phosphate ( $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*



- $C_S$  = concentration of USP Chloroquine Phosphate RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of chloroquine phosphate in the *Sample solution* ( $\mu\text{g/mL}$ )  
 Acceptance criteria: 90.0%–110.0%

**SPECIFIC TESTS**

- **PH** (791): 4.0–5.0

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.
- **BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored in a refrigerator or at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** (11)  
USP Chloroquine Phosphate RS

## Chloroquine Phosphate Tablets

**DEFINITION**

Chloroquine Phosphate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of chloroquine phosphate ( $\text{C}_{18}\text{H}_{26}\text{ClN}_3 \cdot 2\text{H}_3\text{PO}_4$ ).

**IDENTIFICATION**

- **A. ULTRAVIOLET ABSORPTION**

**Standard solution:** 7.5  $\mu\text{g/mL}$  of USP Chloroquine Phosphate RS in water

**Sample solution:** Nominally 7.5  $\mu\text{g/mL}$  of chloroquine phosphate from a filtered solution of finely powdered Tablets in water

**Instrumental conditions**

**Mode:** UV

**Wavelength range:** 329–343 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the absorbance ratio:

$$\text{Result} = A_{343}/A_{329}$$

$A_{343}$  = absorbance of the *Sample solution* at 343 nm

$A_{329}$  = absorbance of the *Sample solution* at 329 nm

**Acceptance criteria:** The peak maxima and minima of the *Sample solution* correspond to those of the *Standard solution*; the absorbance ratio is 1.00–1.15.

- **B.**

**Sample solution:** 20 mL of a filtered 1-mg/mL chloroquine phosphate solution from finely powdered Tablets in water

**Analysis 1:** To the *Sample solution* add 5 mL of trinitrophenol TS.

**Acceptance criteria 1:** A yellow precipitate is formed.

**Analysis 2:** Filter, wash the precipitate with water until the last washing is colorless, and dry over silica gel.

[**CAUTION**—Picrates may explode.]

**Acceptance criteria 2:** The precipitate melts at 205°–210°.

- **C.** The retention time of the chloroquine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- **PROCEDURE**

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Add 1.0 mL of perchloric acid to each 1 L of so-

lution, adjust with phosphoric acid to a pH of 2.5, and pass through a filter of 0.45- $\mu\text{m}$  pore size.

**Mobile phase:** Methanol and *Buffer* (22:78)

**System suitability solution:** 0.15 mg/mL of USP Amodiaquine Hydrochloride RS and 0.15 mg/mL of USP Chloroquine Phosphate RS in water

**Standard solution:** 0.15 mg/mL of USP Chloroquine Phosphate RS in water

**Sample solution:** Nominally 0.15 mg/mL of chloroquine phosphate in water prepared as follows. Transfer nominally 7.5 mg of chloroquine phosphate from finely powdered Tablets (NLT 20) to a 50-mL volumetric flask, and dissolve in and dilute with water to volume. Sonicate for 20 min. Pass 10 mL through a nylon filter of 0.2- $\mu\text{m}$  pore size, discarding the first 4 mL, and use 2 mL for the *Analysis*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 224 nm

**Column:** 4.6-mm  $\times$  10-cm; 5- $\mu\text{m}$  packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 10  $\mu\text{L}$

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for chloroquine phosphate and amodiaquine hydrochloride are 1.0 and 1.3, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between amodiaquine hydrochloride and chloroquine phosphate

**Tailing factor:** NMT 1.5 for the amodiaquine and chloroquine peaks

**Relative standard deviation:** NMT 2.0% for the amodiaquine and chloroquine peaks

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of chloroquine phosphate ( $\text{C}_{18}\text{H}_{26}\text{ClN}_3 \cdot 2\text{H}_3\text{PO}_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Chloroquine Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of chloroquine phosphate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–107.0%

**PERFORMANCE TESTS**

- **DISSOLUTION** (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 100 rpm

**Time:** 45 min

**Detector:** UV

**Analytical wavelength:** 343 nm

**Standard solution:** USP Chloroquine Phosphate RS in *Medium*

**Sample solution:** Dilute with *Medium* to a concentration that is similar to the *Standard solution*.

**Tolerances:** NLT 75% (Q) of the labeled amount of chloroquine phosphate ( $\text{C}_{18}\text{H}_{26}\text{ClN}_3 \cdot 2\text{H}_3\text{PO}_4$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

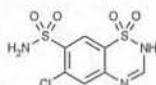
**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.



- **USP REFERENCE STANDARDS (11)**  
USP Amodiaquine Hydrochloride RS  
USP Chloroquine Phosphate RS

## Chlorothiazide



$C_7H_6ClN_3O_4S_2$  295.72

2 *H*-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-, 1,1-dioxide.

6-Chloro-2 *H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [58-94-6].

» Chlorothiazide contains not less than 98.0 percent and not more than 102.0 percent of  $C_7H_6ClN_3O_4S_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

### USP Reference standards (11)—

USP Benzothiadiazine Related Compound A RS  
4-Amino-6-chloro-1,3-benzenedisulfonamide.  
 $C_6H_8ClN_3O_4S_2$  285.73

USP Chlorothiazide RS

### Identification—

**A:** *Infrared Absorption* (197M): previously dried at 105° for 1 hour.

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* 10 µg per mL.

*Medium:* sodium hydroxide solution (1 in 250).

Absorptivities at 292 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Loss on drying** (731)—Dry it at 105° for 1 hour: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chloride**—Dissolve 1.00 g in a mixture of 10 mL of water and 10 mL of sodium hydroxide solution (1 in 10). Cool in an ice bath, and add 20 mL of water and 5 mL of nitric acid. A flocculent, white precipitate is formed. Titrate potentiometrically with 0.050 N silver nitrate, using a silver-silver chloride electrode system: not more than 0.28 mL is required (0.05%).

**Selenium** (291): 0.003%.

### Delete the following:

• **Heavy metals, Method II** (231): 0.001%. • (Official 1-Jan-2018)

### Related compounds—

*Mobile phase, Resolution solution, and Chromatographic system*—Proceed as directed in the Assay.

*Standard solution*—Dissolve an accurately weighed quantity of USP Benzothiadiazine Related Compound A RS in *Mobile phase* to obtain a solution having a known concentration of about 1.5 µg per mL.

*Test solution*—Proceed as directed for Assay preparation in the Assay.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.9 for benzothiadiazine related compound A and 1.0

for chlorothiazide. Calculate the quantity, in mg, of benzothiadiazine related compound A in the portion of Chlorothiazide taken by the formula:

$$0.2C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of USP Benzothiadiazine Related Compound A RS in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses of benzothiadiazine related compound A obtained from the *Test solution* and the *Standard solution*, respectively: not more than 1.0% is present.

### Assay—

*Mobile phase*—Prepare a suitable degassed mixture of 0.1 M monobasic sodium phosphate and acetonitrile (9:1), adjust with phosphoric acid to a pH of  $3.0 \pm 0.1$ , and filter. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Resolution solution*—Dissolve quantities of USP Reference Standards in *Mobile phase* to obtain solutions having known concentrations of about 0.15 mg per mL of USP Chlorothiazide RS and about 1.5 µg per mL of USP Benzothiadiazine Related Compound A RS.

*Standard preparation*—[NOTE—A volume of acetonitrile not exceeding 10% of the total volume of solution may be used to dissolve the reference standard.] Dissolve an accurately weighed quantity of USP Chlorothiazide RS in *Mobile phase* to obtain a solution having a known concentration of about 0.15 mg per mL.

*Assay preparation*—Transfer about 30 mg of Chlorothiazide, accurately weighed, to a 200-mL volumetric flask, dissolve in a small volume of acetonitrile, not exceeding 10% of the total volume of the solution, dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.2 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 1.5%. Chromatograph the *Resolution solution*: the resolution,  $R$ , between benzothiadiazine related compound A and chlorothiazide is not less than 3.5.

*Procedure*—[NOTE—The *Standard preparation* and the *Assay preparation* should be injected immediately upon preparation.] Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major chlorothiazide peaks. The relative retention times are about 0.9 for benzothiadiazine related compound A and 1.0 for chlorothiazide. Calculate the quantity, in mg, of  $C_7H_6ClN_3O_4S_2$  in the portion of Chlorothiazide taken by the formula:

$$200C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorothiazide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of chlorothiazide obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorothiazide Oral Suspension

### DEFINITION

Chlorothiazide Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of chlorothiazide ( $C_7H_6ClN_3O_4S_2$ ).



**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

Solution A: 0.1% formic acid in water  
 Solution B: Methanol  
 Mobile phase: See Table 1.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	94	6
4	94	6
14	80	20
18	40	60
25	40	60
26	94	6
30	94	6

**Diluent:** Acetonitrile and *Solution A* (30:70)

**Standard solution:** 0.1 mg/mL of USP Chlorothiazide RS in *Diluent*

**Sample stock solution:** 0.5 mg/mL of chlorothiazide in *Diluent* prepared as follows. Transfer a suitable amount of Oral Suspension, equivalent to 25 mg of chlorothiazide, to a 50-mL volumetric flask. Add 40 mL of *Diluent* and sonicate to dissolve. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.

**Sample solution:** Nominally 0.1 mg/mL of chlorothiazide in *Diluent*, from *Sample stock solution*

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 275 nm. For *Identification test B*, use a diode array detector in the range of 200–400 nm.

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Temperatures**

**Autosampler:** 5°

**Column:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlorothiazide (C<sub>7</sub>H<sub>6</sub>ClN<sub>3</sub>O<sub>4</sub>S<sub>2</sub>) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of chlorothiazide from the *Sample solution*

$r_S$  = peak response of chlorothiazide from the *Standard solution*

$C_S$  = concentration of USP Chlorothiazide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of chlorothiazide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**• **UNIFORMITY OF DOSAGE UNITS** <905>

For single-unit containers

Acceptance criteria: Meets the requirements

• **DELIVERABLE VOLUME** <698>

For multiple-unit containers

Acceptance criteria: Meets the requirements

**IMPURITIES**• **ORGANIC IMPURITIES**

**Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 10 μg/mL of USP Benzothiadiazine Related Compound A RS and 2 μg/mL of USP Chlorothiazide RS in *Diluent*

**Sample solution:** Nominally 1 mg/mL of chlorothiazide in *Diluent* prepared as follows. Transfer a suitable amount of Oral Suspension, equivalent to 25 mg of chlorothiazide, to a 25-mL volumetric flask. Add 20 mL of *Diluent* and sonicate to dissolve. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.

**System suitability**

**Sample:** *Standard solution*

[NOTE—See Table 2 for relative retention times.]

**Suitability requirements**

**Resolution:** NLT 4.0 between the benzothiadiazine related compound A and chlorothiazide peaks

**Relative standard deviation:** NMT 5.0% for chlorothiazide

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual unspecified degradation product in the portion of Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each unspecified degradation product from the *Sample solution*

$r_S$  = peak response of chlorothiazide from the *Standard solution*

$C_S$  = concentration of USP Chlorothiazide RS in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of chlorothiazide in the *Sample solution* (μg/mL)

Acceptance criteria: See Table 2. Disregard any peak below 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A <sup>a</sup>	0.7	—
Chlorothiazide	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	2.0

<sup>a</sup> Not included in the total degradation products.

**SPECIFIC TESTS**• **pH** <791>: 3.2–4.0**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from freezing and store at controlled room temperature.



• **USP REFERENCE STANDARDS** (11)

USP Benzothiadiazine Related Compound A RS  
4-Amino-6-chloro-1,3-benzenedisulfonamide.  
 $C_6H_6ClN_3O_4S_2$  285.73  
USP Chlorothiazide RS

## Chlorothiazide Tablets

### DEFINITION

Chlorothiazide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of chlorothiazide ( $C_7H_6ClN_3O_4S_2$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

• **PROCEDURE**

**Solution A:** 0.1% formic acid in water  
**Solution B:** Methanol  
**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	94	6
4	94	6
14	80	20
18	40	60
25	40	60
26	94	6
30	94	6

**Diluent:** Acetonitrile and *Solution A* (30:70)

**Standard solution:** 0.1 mg/mL of USP Chlorothiazide RS in *Diluent*

**Sample stock solution:** 0.5 mg/mL of chlorothiazide in *Diluent* prepared as follows. Transfer a suitable amount of finely powdered Tablets (NLT 20), equivalent to 25 mg of chlorothiazide, to a 50-mL volumetric flask. Add 40 mL of *Diluent* and sonicate. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.

**Sample solution:** Nominally 0.1 mg/mL of chlorothiazide in *Diluent* from the *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 275 nm. For *Identification test B*, use a diode array detector in the range of 200–400 nm.

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Temperatures**

**Autosampler:** 5°

**Column:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of chlorothiazide ( $C_7H_6ClN_3O_4S_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of chlorothiazide from the *Sample solution*

$r_S$  = peak response of chlorothiazide from the *Standard solution*

$C_S$  = concentration of USP Chlorothiazide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of chlorothiazide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

• **DISSOLUTION** (711)

**Medium:** 0.05 M pH 8.0 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL

**Apparatus 2:** 75 rpm

**Time:** 60 min

**Standard solution:** Known concentration of USP Chlorothiazide RS in *Medium*

**Sample solution:** Filtered portions of the solution under test, suitably diluted with *Medium* to a concentration that is similar to the *Standard solution*

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** 294 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** NLT 75% (Q) of the labeled amount of chlorothiazide ( $C_7H_6ClN_3O_4S_2$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

### IMPURITIES

• **ORGANIC IMPURITIES**

**Solution A, Solution B, Mobile phase, Diluent, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 10 μg/mL of USP Benzothiadiazine Related Compound A RS and 2 μg/mL of USP Chlorothiazide RS in *Diluent*

**Sample solution:** Nominally 1 mg/mL of chlorothiazide in *Diluent* prepared as follows. Transfer a suitable amount of finely powdered Tablets (NLT 20) to an appropriate volumetric flask. Add *Diluent* to 80% of the final flask volume and sonicate. Dilute with *Diluent* to volume. Centrifuge and use the supernatant.

**System suitability**

**Sample:** *Standard solution*

[NOTE—See Table 2 for relative retention times.]

**Suitability requirements**

**Resolution:** NLT 4.0 between the benzothiadiazine related compound A and chlorothiazide peaks

**Relative standard deviation:** NMT 5.0% for chlorothiazide



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of each unspecified degradation product from the *Sample solution*  
 $r_S$  = peak response of chlorothiazide from the *Standard solution*  
 $C_S$  = concentration of USP Chlorothiazide RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of chlorothiazide in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** See Table 2. Disregard any peak below 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzothiadiazine related compound A*	0.7	—
Chlorothiazide	1.0	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	2.0

\*Not included in the total degradation products.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers. Store at controlled room temperature.
- USP REFERENCE STANDARDS (11)**
  - USP Benzothiadiazine Related Compound A RS
  - 4-Amino-6-chloro-1,3-benzenedisulfonamide.  
 $\text{C}_6\text{H}_6\text{ClN}_3\text{O}_4\text{S}_2$  285.73
  - USP Chlorothiazide RS

**Chlorothiazide Sodium for Injection**

$\text{C}_7\text{H}_5\text{ClN}_3\text{NaO}_4\text{S}_2$  317.71

2H-1,2,4-Benzothiadiazine-7-sulfonamide, 6-chloro-, 1,1-dioxide, monosodium salt.

6-Chloro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide monosodium salt [7085-44-1].

» Chlorothiazide Sodium for Injection is a sterile, freeze-dried mixture of Chlorothiazide Sodium (prepared by the neutralization of Chlorothiazide with the aid of Sodium Hydroxide) and Mannitol. It contains chlorothiazide sodium ( $\text{C}_7\text{H}_5\text{ClN}_3\text{NaO}_4\text{S}_2$ ) equivalent to not less than 93.0 percent and not more than 107.0 percent of the labeled amount of chlorothiazide ( $\text{C}_7\text{H}_6\text{ClN}_3\text{O}_4\text{S}_2$ ).

**Change to read:**

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

**USP Reference standards (11)**—

USP Chlorothiazide RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

**Identification, Ultraviolet Absorption (197U)**—

*Solution:* 10  $\mu\text{g}$  per mL.

*Medium:* sodium hydroxide solution (1 in 250).

**Bacterial Endotoxins Test** (85)—It contains not more than 0.3 USP Endotoxin Unit per mg of chlorothiazide sodium.

**Uniformity of dosage units** (905): meets the requirements.

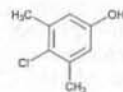
**pH** (791): between 9.2 and 10.0, in a solution prepared as directed in the labeling.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—Transfer an accurately weighed portion of Chlorothiazide Sodium for Injection, equivalent to about 500 mg of chlorothiazide, to a 1000-mL volumetric flask, add sodium hydroxide solution (1 in 250) to volume, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with sodium hydroxide solution (1 in 250) to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Chlorothiazide RS in the same medium having a known concentration of about 10  $\mu\text{g}$  per mL in 1-cm cells at the wavelength of maximum absorbance at about 292 nm, with a suitable spectrophotometer, using sodium hydroxide solution (1 in 250) as the blank. Calculate the quantity, in mg, of  $\text{C}_7\text{H}_6\text{ClN}_3\text{O}_4\text{S}_2$  in the portion of Chlorothiazide Sodium for Injection taken by the formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Chlorothiazide RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the assay solution and the Standard solution, respectively.

**Chloroxylenol**

$\text{C}_8\text{H}_9\text{ClO}$

Phenol, 4-chloro-3,5-dimethyl-

4-Chloro-3,5-xyleneol [88-04-0].

156.61

**DEFINITION**

Chloroxylenol contains NLT 98.5% of chloroxylenol ( $\text{C}_8\text{H}_9\text{ClO}$ ).

**IDENTIFICATION**

- A. INFRARED ABSORPTION (197K)**
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****PROCEDURE**

**Internal standard solution:** 4 mg/mL of USP Parachlorophenol RS in toluene

**Standard solution:** 1 mg/mL of USP Chloroxylenol RS prepared as follows. Transfer 10 mg of USP Chloroxylenol RS to a 10.0-mL volumetric flask, add 2.0 mL of the



*Internal standard solution*, and dilute with toluene to volume.

**Sample solution:** 1 mg/mL of Chloroxylenol prepared as follows. Transfer 10 mg of Chloroxylenol to a 10.0 mL volumetric flask, add 2.0 mL of the *Internal standard solution*, and dilute with toluene to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.32-mm × 30-m; coated with a 0.50-μm film of phase G42

**Temperatures**

**Injection port:** 250°

**Detector:** 250°

**Column:** See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
105	0	105	1
105	6	190	8

**Carrier gas:** Helium

**Flow rate:** 2.4 mL/min

**Injection volume:** 2 μL

**Split ratio:** 25:1

**Run time:** 23 min

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 5.0 between the parachlorophenol peak and the chloroxylenol peak

**Tailing factor:** NMT 1.5 for the chloroxylenol peak

**Relative standard deviation:** NMT 1.5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of chloroxylenol (C<sub>8</sub>H<sub>5</sub>ClO) in the portion of Chloroxylenol taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak area ratio of the chloroxylenol peak to the parachlorophenol peak from the *Sample solution*

$R_S$  = peak area ratio of the chloroxylenol peak to the parachlorophenol peak from the *Standard solution*

$C_S$  = concentration of USP Chloroxylenol RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Chloroxylenol in the *Sample solution* (mg/mL)

**Acceptance criteria:** NLT 98.5%

#### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **IRON** (241)

**Sample:** 0.10 g

**Analysis:** Transfer the *Sample* to a suitable crucible, add 5 drops of sulfuric acid, and ignite at a low heat until thoroughly ashed. Add 10 drops of sulfuric acid to the carbonized mass, and heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace, at 500°–600°, until the carbon is completely burned off. Cool, add 4 mL of 6 N hydrochloric acid, cover, digest on a steam bath for 15 min, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 min. Dilute with water to 25 mL. Filter, if necessary. Rinse the crucible and the filter with 10 mL of water, combining the filtrate and rinsing in a 50-mL color-comparison tube;

add 2 mL of hydrochloric acid; dilute with water to 47 mL; and mix.

**Acceptance criteria:** NMT 0.01%

#### • LIMIT OF TETRACHLOROETHYLENE

**Internal standard stock solution:** 20 μL/mL of 1-butanol in methanol

**Internal standard solution:** 2 μL/mL of 1-butanol in methanol from *Internal standard stock solution*

**Tetrachloroethylene standard stock solution:** 20 μL/mL of tetrachloroethylene in methanol

**Tetrachloroethylene standard solution:** 2 μL/mL of tetrachloroethylene in methanol from *Tetrachloroethylene standard stock solution*

**Standard solution:** 0.4 μL/mL each of 1-butanol and tetrachloroethylene in methanol from *Internal standard solution* and *Tetrachloroethylene standard solution*, respectively, prepared as follows. Combine 5 mL each of *Internal standard solution* and *Tetrachloroethylene standard solution* in a 25-mL volumetric flask, dilute with methanol to volume, and mix.

**Sample solution:** 160 mg/mL of chloroxylenol and 0.4 μL/mL of 1-butanol in methanol prepared as follows. Weigh 4 g of chloroxylenol in a 25-mL volumetric flask, combine with 5 mL of *Internal standard solution*, and dilute with methanol to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm × 30-m; 1.0-μm film of phase G14 or G16

**Carrier gas:** Hydrogen

**Temperatures**

**Injector:** 240°

**Detector:** 240°

**Column:** See Table 2.

Table 2

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
70	0	70	2
70	35	210	5

**Flow rate:** 12.8 mL/min

**Injection volume:** 0.5 μL

**Split ratio:** 20:1

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for tetrachloroethylene and 1-butanol are about 1.0 and 1.9, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between tetrachloroethylene and the solvent front of methanol

**Tailing factor:** NMT 1.2 for the tetrachloroethylene and 1-butanol peaks

**Relative standard deviation:** NMT 8.0% for the ratio of the 1-butanol to the tetrachloroethylene peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of tetrachloroethylene in the portion of Chloroxylenol taken by comparing the peak response ratio of tetrachloroethylene to the internal standard from the *Standard solution* to that of the peak response ratio of tetrachloroethylene to the internal standard from the *Sample solution*:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times d \times F \times 100$$

$R_U$  = peak response ratio of tetrachloroethylene to 1-butanol from the *Sample solution*



- $R_S$  = peak response ratio of tetrachloroethylene to 1-butanol from the *Standard solution*  
 $C_S$  = concentration of tetrachloroethylene in the *Standard solution* (mL/mL)  
 $C_U$  = concentration of Chloroxylenol in the *Sample solution* (mg/mL)  
 $d$  = density of tetrachloroethylene, 1.623 g/mL  
 $F$  = conversion factor, 1 mg/0.001 g

Acceptance criteria: NMT 0.4% of tetrachloroethylene

#### • ORGANIC IMPURITIES

**Standard solution:** 0.02 mg/mL each of 3,5-dimethylphenol and USP Chloroxylenol Related Compound A RS in toluene

**Sample solution:** 10.0 mg/mL of Chloroxylenol in toluene

**Chromatographic system:** Proceed as directed in the *Assay*.

#### System suitability

**Sample:** *Standard solution*

[NOTE—For relative retention times, see *Table 3*.]

#### Suitability requirements

**Resolution:** NLT 8 between 3,5-dimethylphenol and chloroxylenol related compound A

**Relative standard deviation:** NMT 3%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentages of 3,5-dimethylphenol ( $C_8H_{10}O$ ) and chloroxylenol related compound A ( $C_8H_9ClO$ ) in the portion of Chloroxylenol taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of the appropriate analyte from the *Sample solution*  
 $r_S$  = peak response of the 3,5-dimethylphenol or chloroxylenol related compound A from the *Standard solution*  
 $C_S$  = concentration of 3,5-dimethylphenol or USP Chloroxylenol Related Compound A RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of the *Sample solution* (mg/mL)  
 Calculate the percentage of each unspecified impurity in the portion of Chloroxylenol taken:

$$\text{Result} = (r_U/r_T) \times 100$$

- $r_U$  = peak response of each unspecified impurity from the *Sample solution*  
 $r_T$  = sum of all the peak responses  
 Acceptance criteria: See *Table 3*.

**Table 3**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
3,5-Dimethylphenol	0.58	0.2
Chloroxylenol related compound A	0.64	0.2
Chloroxylenol	1.0	—
Any individual impurity	—	0.5
Total impurities	—	1.5

#### SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 0.5%

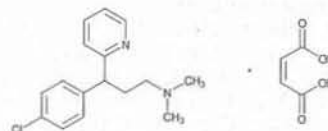
#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

#### • USP REFERENCE STANDARDS (11)

- USP Chloroxylenol RS  
 USP Chloroxylenol Related Compound A RS  
 2-Chloro-3,5-dimethylphenol.  
 USP Parachlorophenol RS

## Chlorpheniramine Maleate



$C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  390.86  
 2-Pyridinepropanamine,  $\gamma$ -(4-chlorophenyl)-N,N-dimethyl-, (Z)-2-butenedioate (1:1);  
 2-[p-Chloro- $\alpha$ -[2-(dimethylamino)ethyl]benzyl] pyridine maleate (1:1) [113-92-8].

#### DEFINITION

Chlorpheniramine Maleate contains NLT 98.0% and NMT 102.0% of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**  
 • **B.** The retention times of the maleic acid and chlorpheniramine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### • PROCEDURE

**Solution A:** 5.44 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.

**Solution B:** Acetonitrile

**Diluent:** Acetonitrile and *Solution A* (5:95)

**System suitability stock solution:** 0.02 mg/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Related Compound B RS, and USP Chlorpheniramine Related Compound C RS in *Diluent*. Sonicate for 1 min.

**System suitability solution:** 0.5 mg/mL of USP Chlorpheniramine Maleate RS and 2  $\mu$ g/mL each of USP Pheniramine Maleate RS, USP Chlorpheniramine Related Compound B RS, and USP Chlorpheniramine Related Compound C RS in *Diluent*, prepared as follows. Transfer 5.0 mg of USP Chlorpheniramine Maleate RS to a 10-mL volumetric flask, add 5 mL of *Diluent* and 1.0 mL of the *System suitability stock solution*, and dilute with *Diluent* to volume. Sonicate for 1 min.

**Standard solution:** 0.5 mg/mL of USP Chlorpheniramine Maleate RS in *Diluent*. Sonicate for 1 min.

**Sample solution:** 0.5 mg/mL of Chlorpheniramine Maleate in *Diluent*. Sonicate for 1 min.

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	95	5
1	95	5
20	70	30
30	70	30
31	95	5
40	95	5



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 225 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 μL

**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements**

[NOTE—The relative retention times of maleic acid, chlorpheniramine related compound C, and chlorpheniramine are 0.18, 0.94 and 1.0, respectively.]

**Resolution:** NLT 1.5 between chlorpheniramine related compound C and chlorpheniramine; and NLT 2.0 between chlorpheniramine related compound B and pheniramine, *System suitability solution***Tailing factor:** NMT 2.0 for chlorpheniramine, *Standard solution***Relative standard deviation:** NMT 0.73% for chlorpheniramine, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Chlorpheniramine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of chlorpheniramine from the *Sample solution* $r_S$  = peak response of chlorpheniramine from the *Standard solution* $C_S$  = concentration of USP Chlorpheniramine Maleate RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Chlorpheniramine Maleate in the *Sample solution* (mg/mL)**Acceptance criteria:** 98.0%–102.0% on the dried basis**IMPURITIES**• **RESIDUE ON IGNITION** (281): NMT 0.2%• **ORGANIC IMPURITIES***Solution A*, *Solution B*, *Diluent*, *System suitability solution*, *Mobile phase*, and *Chromatographic system*: Proceed as directed in the *Assay*.**Standard solution:** 1.4 μg/mL of USP Chlorpheniramine Maleate RS in *Diluent*, equivalent to 1.0 μg/mL of chlorpheniramine. Sonicate for 1 min.**Sensitivity solution:** 0.28 μg/mL of USP Chlorpheniramine Maleate RS in *Diluent***Sample solution:** 0.5 mg/mL of Chlorpheniramine Maleate in *Diluent*. Sonicate for 1 min.**System suitability****Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution***Suitability requirements****Resolution:** NLT 1.5 between chlorpheniramine related compound C and chlorpheniramine; and NLT 2.0 between the chlorpheniramine related compound B and pheniramine, *System suitability solution***Relative standard deviation:** NMT 5.0% for chlorpheniramine, *Standard solution***Signal-to-noise ratio:** NLT 10 for chlorpheniramine, *Sensitivity solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Chlorpheniramine Maleate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution* $r_S$  = peak response of chlorpheniramine from the *Standard solution* $C_S$  = concentration of chlorpheniramine from the *Standard solution* (mg/mL) $C_U$  = concentration of Chlorpheniramine Maleate in the *Sample solution* (mg/mL) $F$  = relative response factor (see *Table 2*)**Acceptance criteria:** See *Table 2*. Disregard peaks having areas less than 0.05% that of chlorpheniramine.**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Maleic acid <sup>a</sup>	0.18	—	—
Diamine analog <sup>b</sup>	0.37	0.73	0.2
Chlorpheniramine related compound B <sup>c</sup>	0.49	0.77	0.1
Pheniramine <sup>d</sup>	0.57	—	—
Chlorpheniramine related compound C <sup>e</sup>	0.97	1.0	0.1
Chlorpheniramine	1.0	—	—
Chlorpheniramine nitrile <sup>f</sup>	1.19	1.0	0.1
Any other unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.5

<sup>a</sup> Salt counter ion is included in the table for identification purposes only.<sup>b</sup> 2-(4-Chlorophenyl)-4-(dimethylamino)-2-[2-(dimethylamino)ethyl]butanenitrile.<sup>c</sup> Di(pyridin-2-yl)amine.<sup>d</sup> Used only to establish the system suitability.<sup>e</sup> 3-(4-Chlorophenyl)-N-methyl-3-(pyridin-2-yl)propan-1-amine.<sup>f</sup> 2-(4-Chlorophenyl)-4-(dimethylamino)-2-(pyridin-2-yl)butanenitrile.**SPECIFIC TESTS**• **OPTICAL ROTATION** (781)**Sample:** 100 mg/mL in water at 20°**Acceptance criteria:** −0.10° to +0.10°• **LOSS ON DRYING** (731)**Analysis:** Dry at 105° for 3 h.**Acceptance criteria:** NMT 0.5%**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.• **USP REFERENCE STANDARDS** (11)

USP Chlorpheniramine Maleate RS

USP Chlorpheniramine Related Compound B RS

Di(pyridin-2-yl)amine.

 $C_{10}H_9N_3$  171.20

USP Chlorpheniramine Related Compound C RS

3-(4-Chlorophenyl)-N-methyl-3-(pyridin-2-yl)propan-1-amine.

 $C_{15}H_{17}ClN_2$  260.76

USP Pheniramine Maleate RS

**Chlorpheniramine Maleate Extended-Release Capsules**» Chlorpheniramine Maleate Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ).



**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label the Capsules to indicate the *Dissolution Test* with which the product complies.

**USP Reference standards (11)**—

USP Chlorpheniramine Maleate RS

**Identification**—

**A:** The retention time of the chlorpheniramine peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**B:** Transfer the contents of 1 Capsule to a 10-mL volumetric flask, add 5 mL of methanol, and insert the stopper into the flask. Sonicate this solution for 10 minutes, dilute with water to volume, mix, and filter. Apply separately 10  $\mu$ L of this solution and 10  $\mu$ L of a solution of USP Chlorpheniramine Maleate RS in a mixture of methanol and water (1:1) containing about 1.2 mg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of ethyl acetate, methanol, and ammonium hydroxide (100:5:5) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength UV light: the  $R_f$  value of the principal spot observed in the chromatogram of the solution under test corresponds to that obtained from the Standard solution.

**Dissolution (711)**—

**TEST 1**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

**Medium:** water; 500 mL.

**Apparatus 1:** 100 rpm.

**Times:** 1.5, 6.0, and 10.0 hours.

**Procedure**—Determine the amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  dissolved by employing the method set forth in the *Assay*, using a filtered portion of the solution under test in comparison with a Standard solution having a known concentration of USP Chlorpheniramine Maleate RS in the same medium.

**Tolerances**—The percentages of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1.5	between 15% and 40%
6.0	between 50% and 80%
10.0	not less than 70%

**TEST 2**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*. Proceed as directed for *Procedure* for *Method B* under *Apparatus 1* and *Apparatus 2, Delayed-Release Dosage Forms*.

**Medium**—Prepare as directed under *Method B*, except use 900 mL of media. Operate the apparatus for 1 hour in the *Acid Stage* and use the acceptance criteria given under *Tolerances*. Operate the apparatus for 6 hours in the *Buffer Stage*, except to use 900 mL of simulated intestinal fluid TS without enzyme, and use the acceptance criteria given under *Tolerances*.

**Apparatus 2:** 50 rpm.

**Times:** 1.0 hour, 3.0 hours, 7.0 hours.

**Procedure**—Proceed as directed in *Test 1*.

**Tolerances**—The percentages of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  dissolved at the times specified conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
1.0	between 30% and 60%
3.0	between 55% and 85%
7.0	not less than 70%

**Uniformity of dosage units (905)**—meet the requirements.

**Assay**—

**Mobile phase**—Dissolve 2.0 g of sodium perchlorate in 350 mL of water. Add 650 mL of methanol and 2.0 mL of triethylamine, and mix. Filter, and degas this solution prior to use. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in dilute hydrochloric acid (1 in 100) to obtain a solution having a known concentration of about 0.12 mg per mL.

**Assay preparation**—Weigh and mix the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the mixture, equivalent to about 120 mg of chlorpheniramine maleate, to a 200-mL volumetric flask. Add about 100 mL of dilute hydrochloric acid (1 in 100), bring to a boil on a hot plate, and continue boiling moderately for 5 minutes. Cool, dilute with dilute hydrochloric acid (1 in 100) to volume, mix, and filter. Transfer 10.0 mL of the filtrate to a 50-mL volumetric flask, dilute with dilute hydrochloric acid (1 in 100) to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 261-nm detector and a 3.9-mm  $\times$  15-cm column that contains 10- $\mu$ m packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 900 theoretical plates, the tailing factor is not greater than 2.0, and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Capsules taken by the formula:

$$(1000C)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorpheniramine Maleate Injection

» Chlorpheniramine Maleate Injection is a sterile solution of Chlorpheniramine Maleate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards (11)**—

USP Chlorpheniramine Maleate RS

USP Endotoxin RS

**Identification**—

**A:** Dilute a volume of Injection, equivalent to about 50 mg of chlorpheniramine maleate, with dilute hydrochloric



ric acid (1 in 1000) to 25 mL, and proceed as directed under *Identification—Organic Nitrogenous Bases* (181), beginning with "Transfer the liquid to a separator." The Injection meets the requirements of the test.

**B:** Evaporate a volume of Injection, equivalent to about 25 mg of chlorpheniramine maleate, on a steam bath to dryness, and dry the residue at 105° for 1 hour: it melts between 128° and 135°.

**Bacterial Endotoxins Test** (85)—It contains not more than 8.8 USP Endotoxin Units per mg of chlorpheniramine maleate.

**pH** (791): between 4.0 and 5.2.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—Proceed with Injection as directed under *Salts of Organic Nitrogenous Bases* (501), to prepare the solution employed for the determination of the absorbance,  $A_U$ , at 264 nm. For the determination of  $A_S$ , dissolve about 25 mg of USP Chlorpheniramine Maleate RS, accurately weighed, in 20 mL of dilute sulfuric acid (1 in 350), and treat this solution the same as the portion of Injection being assayed. Calculate the quantity, in mg, of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  in each mL of the Injection taken by the formula:

$$(C/V)(A_U/A_S)$$

in which C is the weight, in mg, of USP Chlorpheniramine Maleate RS in the *Standard preparation*, and V is the volume, in mL, of Injection taken.

## Chlorpheniramine Maleate Oral Solution

» Chlorpheniramine Maleate Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Chlorpheniramine Maleate RS

**Identification**—

**A:** Evaporate the remaining extract from the *Assay* on a steam bath to a small volume, then transfer it to a smaller, more suitable vessel, and evaporate just to the point where hexane vapors are no longer perceptible. Transfer the oily residue, with the aid of four 3-mL portions of dimethylformamide, to a suitable glass-stoppered graduated cylinder, dilute with dimethylformamide to 15.0 mL, and mix: the optical rotation of the solution so obtained, in a 100-mm tube, after correcting for the blank, is not more than +0.01° (*distinction from dexchlorpheniramine maleate*).

**B:** *Ultraviolet Absorption* (197U)—

*Solution:* the solution employed for measurement of absorbance in the *Assay*.

**Alcohol Determination** (611) (*if present*) (611): between 6.0% and 8.0% of  $C_2H_5OH$ .

**Assay**—Transfer 10 mL of Oral Solution, accurately measured, to a separator. Transfer about 40 mg of USP Chlorpheniramine Maleate RS, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, mix, and pipet 10 mL of this Standard solution into a separator similar to that containing the Oral Solution. Treat each solution as follows. Add 10 mL of sodium hydroxide solution (1 in 10), and extract with two 50-mL portions of solvent hexane: Combine the extracts in a second separator, wash with 10 mL of sodium hydroxide solution (1 in 250), and discard

the washing. Extract the hexane solution with two 40-mL portions of dilute hydrochloric acid (1 in 100), collect the extracts in a 100-mL volumetric flask, add the same dilute acid to volume, and mix. Wash 50-mL portions of each solution, and of dilute hydrochloric acid (1 in 100), respectively, with three 30-mL portions of chloroform and then with 50 mL of solvent hexane, and discard the washings. Filter the acid phases through paper, discarding the first few mL of each filtrate, and determine the absorbances of the solutions obtained from the Oral Solution and the Standard solution in 1-cm cells at the wavelength of maximum absorbance at about 264 nm, with a suitable spectrophotometer, using the extracted acid as the blank. Calculate the quantity, in  $\mu$ g, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in each mL of the Oral Solution taken by the formula:

$$C(A_U/A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Chlorpheniramine Maleate RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the Oral Solution and the Standard solution, respectively.

## Chlorpheniramine Maleate Tablets

» Chlorpheniramine Maleate Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Chlorpheniramine Maleate RS

**Identification**—Disperse a portion of powdered Tablets, equivalent to about 25 mg of chlorpheniramine maleate, in about 20 mL of dilute hydrochloric acid (1 in 100). Dissolve about 25 mg of USP Chlorpheniramine Maleate RS in 20 mL of dilute hydrochloric acid (1 in 100). Treat each solution as follows. Render alkaline, to a pH of about 11, with sodium hydroxide solution (1 in 10). Extract with two 50-mL portions of solvent hexane, collect the extracts in a beaker, and evaporate to dryness. Prepare a mineral oil dispersion of the residue so obtained and determine the IR absorption spectrum of the preparation in the region between 2  $\mu$ m and 12  $\mu$ m: the spectrum of the test preparation exhibits maxima only at the same wavelengths as that of the Standard preparation.

**Dissolution** (711)—

*Medium:* 0.01 N hydrochloric acid; 500 mL

*Apparatus 2:* 50 rpm.

*Time:* 30 minutes.

*Procedure*—Determine the amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 265 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Chlorpheniramine Maleate RS in the same *Medium*.

*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—Using a portion of powdered Tablets equivalent to 4 mg of chlorpheniramine maleate, proceed as directed under *Salts of Organic Nitrogenous Bases* (501), but using dilute hydrochloric acid (1 in 100) instead of the dilute sulfuric acid (1 in 350), and dilute sulfuric acid (1 in 70), and



using solvent hexane instead of the ether, and diluting 10 mL of the *Assay preparation* with dilute hydrochloric acid (1 in 100) to 25.0 mL to prepare the solution employed for the determination of the absorbance,  $A_U$ , at 264 nm. For the determination of  $A_S$ , prepare a solution containing about 40 mg of USP Chlorpheniramine Maleate RS, accurately weighed, in 200.0 mL of dilute hydrochloric acid (1 in 100), and treat 20.0 mL of this solution the same as the solution in dilute hydrochloric acid (1 in 100) of the portion of Tablets taken. Calculate the quantity, in mg, of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  in the portion of Tablets taken by the formula:

$$C(A_U / A_S)$$

in which C is the weight, in mg, of USP Chlorpheniramine Maleate RS in the 20.0-mL portion of the *Standard preparation*.

### Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules

» Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature.

**Labeling**—The labeling indicates the *Dissolution Test* with which the product complies.

**USP Reference standards** (11)—

USP Chlorpheniramine Maleate RS  
USP Pseudoephedrine Hydrochloride RS

**Identification**—

**A:** The retention time of the major peak for chlorpheniramine maleate in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.

**B:** The retention time of the major peak for pseudoephedrine hydrochloride in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay for pseudoephedrine hydrochloride*.

**Dissolution** (711)—

**TEST 1**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Times:* 3, 6, and 12 hours.

**Procedure**—Determine the amounts of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) dissolved by employing the methods set forth in the *Assay for chlorpheniramine maleate* and the *Assay for pseudoephedrine hydrochloride*, respectively.

**Tolerances**—The percentages of the labeled amounts of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  and  $C_{10}H_{15}NO \cdot HCl$  dissolved at the specified times conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved
3	between 20% and 50%
6	between 45% and 75%
12	not less than 75%

**TEST 2**—If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

*Medium 1:* simulated gastric fluid TS, prepared without pepsin; 900 mL.

*Medium 2:* simulated intestinal fluid TS, prepared without pancreatin; 900 mL.

*Apparatus 2:* 50 rpm.

*Time for Medium 1:* 1.5 hours.

*Times for Medium 2:* 3 and 6 hours.

**Procedure**—Determine the amounts of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) dissolved by employing the methods set forth in the *Assay for chlorpheniramine maleate* and the *Assay for pseudoephedrine hydrochloride*, respectively, using Standard solutions having known concentrations of the relevant USP Reference Standard in the appropriate *Medium*.

**Tolerances**—The percentages of the labeled amounts of  $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$  and  $C_{10}H_{15}NO \cdot HCl$  dissolved at the specified times conform to *Acceptance Table 2*.

Time (hours)	Amount dissolved (Medium 1)	Amount dissolved (Medium 2)
1.5	between 15% and 40%	
3.0		between 35% and 75%
6.0		not less than 50%

**Uniformity of dosage units** (905): meet the requirements.

**Assay for chlorpheniramine maleate**—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (60:40) containing 0.34 g of monobasic potassium phosphate, 0.15 g of triethylamine hydrochloride, 0.25 g of sodium lauryl sulfate, and 0.1 mL of phosphoric acid in each 100 mL of solution. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 0.8 mg per mL. Quantitatively dilute a portion of this solution with a phosphoric acid solution (1 in 1000) to obtain a solution having a known concentration of about 8 µg per mL.

**Assay preparation**—Transfer not fewer than 10 Capsules to a suitable container. Add 100 mL of water and 10 mL of a phosphoric acid solution (1 in 20), and heat gently until the Capsules are fully dispersed. Cool to room temperature, and transfer an accurately measured volume of the solution, equivalent to about 0.8 mg of chlorpheniramine maleate, to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter.

**System suitability solution**—Mix 1 part of the *Standard preparation* prepared above with 1 part of the *Standard preparation*, prepared as directed in the *Assay for pseudoephedrine hydrochloride*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a 4.6-mm × 15-cm column that contains packing L11. The flow rate is about 2 mL per minute. Inject about 20 µL of the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between pseudoephedrine and chlorpheniramine is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor



for the chlorpheniramine peak is not greater than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Capsules taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Chlorpheniramine Maleate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for pseudoephedrine hydrochloride—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay for chlorpheniramine maleate*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 3.0 mg per mL. Transfer about 1.0 mL of this solution to a 25-mL volumetric flask, dilute with 0.1% phosphoric acid to volume, and mix.

**System suitability solution**—Mix 1 part of the *Standard preparation* prepared above with 1 part of the *Standard preparation* prepared as directed in the *Assay for chlorpheniramine maleate*.

**Assay preparation**—Transfer not fewer than 10 Capsules to a suitable container. Add 100 mL of water and 10 mL of a phosphoric acid solution (1 in 20), and heat gently until the Capsules are fully dispersed. Cool to room temperature, and transfer an accurately measured volume of the solution, equivalent to about 12 mg of pseudoephedrine hydrochloride, to a 100-mL volumetric flask. Dilute with water to volume, mix, and filter.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Capsules taken by the formula:

$$100C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Oral Solution

» Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amounts of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) and pseudoephedrine hydrochloride ( $C_{10}H_{15}NO \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Chlorpheniramine Maleate RS  
USP Pseudoephedrine Hydrochloride RS

#### Identification—

**A:** The retention time of the major peak for chlorpheniramine maleate in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, as obtained in the *Assay for chlorpheniramine maleate*.

**B:** The retention time of the major peak for pseudoephedrine hydrochloride in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation* in the *Assay for pseudoephedrine hydrochloride*.

#### Uniformity of dosage units (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

#### Deliverable volume (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

#### Assay for chlorpheniramine maleate—

**Mobile phase, System suitability solution, and Chromatographic system**—Proceed as directed in the *Assay for chlorpheniramine maleate* under *Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpheniramine Maleate RS in water to obtain a solution having a known concentration of about 1 mg per mL. Transfer 1.0 mL of this solution to a 100-mL volumetric flask, add 80 mL of *Mobile phase*, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 1 mg of chlorpheniramine maleate, to a 100-mL volumetric flask. Add about 80 mL of *Mobile phase*, dilute with water to volume, mix, and filter.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the chlorpheniramine peaks. Calculate the quantity, in mg, of chlorpheniramine maleate ( $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ ) in the portion of Oral Solution taken by the formula:

$$(100C/V)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpheniramine Maleate RS in that *Standard preparation*; V is the volume, in mL, of Oral Solution taken for the *Assay preparation*; and  $r_U$  and  $r_S$  are the chlorpheniramine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

#### Assay for pseudoephedrine hydrochloride—

**Mobile phase, System suitability solution, and Chromatographic system**—Proceed as directed in the *Assay for chlorpheniramine maleate* under *Chlorpheniramine Maleate and Pseudoephedrine Hydrochloride Extended-Release Capsules*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Pseudoephedrine Hydrochloride RS in water to obtain a solution having a known concentration of about 1.5 mg per mL. Transfer about 1.0 mL of this solution to a 10-mL volumetric flask, add 8 mL of *Mobile phase*, dilute with water to volume, and mix.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 15 mg of pseudoephedrine hydrochloride, to a 100-mL volumetric flask. Add 80 mL of *Mobile phase*, dilute with water to volume, mix, and filter.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the pseudoephedrine peaks. Calculate the quantity, in mg, of pseudoephedrine hydrochloride

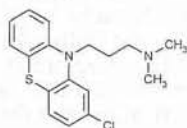


( $C_{10}H_{15}NO \cdot HCl$ ) in the portion of Oral Solution taken by the formula:

$$100(C/V)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Pseudoephedrine Hydrochloride RS in the *Standard preparation*;  $V$  is the volume, in mL, of Oral Solution taken for the *Assay preparation*; and  $r_U$  and  $r_S$  are the pseudoephedrine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorpromazine



$C_{17}H_{19}ClN_2S$  318.86

10*H*-Phenothiazine-10-propanamine, 2-chloro-*N,N*-dimethyl-

2-Chloro-10-[3-(dimethylamino)propyl]phenothiazine [50-53-3].

» Chlorpromazine contains not less than 98.0 percent and not more than 101.0 percent of  $C_{17}H_{19}ClN_2S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

### USP Reference standards (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

### Identification—

**A:** The IR absorption spectrum of a 1 in 100 solution in carbon disulfide, in a 1.0-mm cell between 7  $\mu$ m and 15  $\mu$ m, exhibits maxima only at the same wavelengths as that of a solution prepared by dissolving 55 mg of USP Chlorpromazine Hydrochloride RS in 3 mL of 1 N sodium hydroxide and extracting the resulting solution with 5.0 mL of carbon disulfide.

**B:** The principal spot found in the test for *Other alkylated phenothiazines* corresponds in  $R_f$  to the spot from the *Standard solution*.

**Loss on drying** (731)—Dry it in vacuum at room temperature for 3 hours: it loses not more than 1.0% of its weight.

**Other alkylated phenothiazines**—Dissolve 45.0 mg in 10 mL of methanol. Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS in methanol to obtain a concentration of 5 mg per mL (*Standard solution*), and dilute it quantitatively and stepwise with methanol to obtain a concentration of 25  $\mu$ g per mL (*Diluted standard solution*). Apply separately 10  $\mu$ L of each of the three solutions to the starting line of a thin-layer chromatographic plate coated with chromatographic silica gel mixture. Develop the chromatogram, using as the solvent system a freshly prepared mixture of equal volumes of ether and ethyl acetate saturated with ammonium hydroxide, until the solvent front has moved about 10 cm from the origin. Remove the plate from the chamber, and air-dry for 20 minutes. View under short-wavelength UV light: the area and intensity of any spot, other than the principal spot, from the solution of Chlorpromazine are not greater than those of the spot from the *Diluted standard solution* (0.5%).

**Assay**—Place about 750 mg of Chlorpromazine, accurately weighed, in a 250-mL conical flask, and dissolve in 25 mL of glacial acetic acid, warming gently on a steam bath to effect solution. Cool, add crystal violet TS, and titrate with 0.1 N perchloric acid VS to a blue endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 31.89 mg of  $C_{17}H_{19}ClN_2S$ .

## Chlorpromazine Suppositories

» Chlorpromazine Suppositories contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{17}H_{19}ClN_2S$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers, at controlled room temperature.

### USP Reference standards (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

**Identification**—Suppositories respond to *Identification test B* under *Chlorpromazine*.

**Other alkylated phenothiazines**—Transfer a portion of Suppositories, equivalent to 45 mg of chlorpromazine, to a stoppered centrifuge tube, add 10 mL of methanol, shake vigorously to disperse the solid, warming gently if necessary, and centrifuge. Proceed as directed in the test for *Other alkylated phenothiazines* under *Chlorpromazine*, beginning with "Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS." The area and intensity of any spot, other than the principal spot, from the solution from the Suppositories are not greater than those of the spot from the *Diluted standard solution* (0.5%).

**Assay**—Place not fewer than 10 Suppositories in a 250-mL beaker, reduce the mass to the consistency of a paste by crushing with a spatula, and mix. Weigh accurately a portion of the mass, equivalent to about 50 mg of chlorpromazine, place in a beaker, and dissolve in about 40 mL of ether. Transfer to a 250-mL separator with the aid of three 25-mL portions of ether, and extract with four 75-mL portions of 0.1 N hydrochloric acid, collecting the aqueous extracts in a 500-mL volumetric flask. Add 0.1 N hydrochloric acid to volume, and mix. Transfer 10.0 mL of this solution to a 200-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Dissolve an accurately weighed quantity of USP Chlorpromazine Hydrochloride RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a *Standard solution* having a known concentration of about 5.5  $\mu$ g of chlorpromazine hydrochloride per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 254 nm and at 277 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of chlorpromazine ( $C_{17}H_{19}ClN_2S$ ) in the portion of Suppositories taken by the formula:

$$10(0.897C)(A_{254} - A_{277})_U / (A_{254} - A_{277})_S$$

in which 0.897 is the ratio of the molecular weight of chlorpromazine to that of chlorpromazine hydrochloride;  $C$  is the concentration, in  $\mu$ g per mL, of USP Chlorpromazine Hydrochloride RS in the *Standard solution*; and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Suppositories ( $U$ ) and the *Standard solution* ( $S$ ), respectively.



**Chlorpromazine Hydrochloride**C<sub>17</sub>H<sub>19</sub>ClN<sub>2</sub>S · HCl 355.3310*H*-Phenothiazine-10-propanamine, 2-chloro-*N,N*-dimethyl-, monohydrochloride.

2-Chloro-10-[3-(dimethylamino)propyl]phenothiazine monohydrochloride [69-09-0].

» Chlorpromazine Hydrochloride contains not less than 98.0 percent and not more than 101.5 percent of C<sub>17</sub>H<sub>19</sub>ClN<sub>2</sub>S · HCl, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

**Identification**—

**A:** Infrared Absorption (197K).

**B:** The principal spot found in the test for *Other alkylated phenothiazines* corresponds in *R<sub>f</sub>* to the spot from the *Standard solution*.

**C:** A solution (1 in 10) responds to the tests for *Chloride* (191).

**Melting range** (741): between 195° and 198°.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Other alkylated phenothiazines**—Dissolve 50 mg, previously dried, in methanol to make 10 mL, and mix. Proceed as directed in the test for *Other alkylated phenothiazines* under *Chlorpromazine*, beginning with "Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS." The area and intensity of any spot, other than the principal spot, from the solution of Chlorpromazine Hydrochloride are not greater than those of the spot from the *Diluted standard solution* (0.5%).

**Assay**—Transfer to a beaker about 700 mg of Chlorpromazine Hydrochloride, accurately weighed, and dissolve in 75 mL of glacial acetic acid. Add 10 mL of mercuric acetate TS, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Each mL of 0.1 N perchloric acid is equivalent to 35.53 mg of C<sub>17</sub>H<sub>19</sub>ClN<sub>2</sub>S · HCl.

**Chlorpromazine Hydrochloride Oral Concentrate**

» Chlorpromazine Hydrochloride Oral Concentrate contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>17</sub>H<sub>19</sub>ClN<sub>2</sub>S · HCl.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate that it must be diluted prior to administration.

**USP Reference standards** (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

**Identification**—

**A:** It responds to *Identification test A* under *Chlorpromazine Hydrochloride Syrup*.

**B:** Dilute a portion of the Oral Concentrate with an equal volume of water: the resulting solution responds to the tests for *Chloride* (191).

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for the absence of *Escherichia coli*.

**pH** (791): between 2.3 and 4.1.

**Limit of chlorpromazine sulfoxide**—Proceed as directed in the test for *Chlorpromazine sulfoxide* under *Chlorpromazine Hydrochloride Syrup*.

**Assay**—Transfer an accurately measured volume of Oral Concentrate, previously diluted if necessary, equivalent to about 10 mg of chlorpromazine hydrochloride, to a 50-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Proceed as directed in the *Assay* under *Chlorpromazine Hydrochloride Injection*, beginning with "Pipet 10 mL of the solution." Calculate the quantity, in mg, of C<sub>17</sub>H<sub>19</sub>ClN<sub>2</sub>S · HCl in each mL of the Oral Concentrate taken by the formula:

$$1.25C(A_{254} - A_{277})_U / V(A_{254} - A_{277})_S$$

in which C is the concentration, in µg per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution; V is the volume, in mL, of Oral Concentrate taken; and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Oral Concentrate (U) and the Standard solution (S), respectively.

**Chlorpromazine Hydrochloride Injection**

» Chlorpromazine Hydrochloride Injection is a sterile solution of Chlorpromazine Hydrochloride in Water for Injection. It contains, in each mL, not less than 23.75 mg and not more than 26.25 mg of C<sub>17</sub>H<sub>19</sub>ClN<sub>2</sub>S · HCl.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Chlorpromazine Hydrochloride RS

USP Endotoxin RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

**Identification**—

**A:** Transfer a volume of Injection, equivalent to about 25 mg of chlorpromazine hydrochloride, to a 10-mL volumetric flask, dilute with methanol to volume, and mix (test solution). Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS in dilute methanol (9 in 10) to obtain a Standard solution having a known concentration of 2.5 mg per mL. Apply separately 5-µL portions of each of the two solutions to the starting line of a thin-layer chromatographic plate (see *Chromatography* (621)) coated with chromatographic silica gel mixture. Develop the chromatogram in a solvent system consisting of a freshly prepared mixture of equal volumes of ether and ethyl acetate saturated with ammonium hydroxide until the solvent front has moved about 10 cm from the origin. Remove the plate from the developing chamber, air-dry for 20 minutes, then view



under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**B:** It responds to the tests for *Chloride* (191).

**Bacterial Endotoxins Test** (85)—It contains not more than 6.9 USP Endotoxin Units per mg of chlorpromazine hydrochloride.

**pH** (791): between 3.4 and 5.4.

**Limit of chlorpromazine sulfoxide**—[NOTE—Conduct this test without exposure to daylight, and with the minimum necessary exposure to artificial light.]

**Test preparation**—Pipet 4 mL of the test solution prepared with methanol as directed in *Identification* test A into a 10-mL volumetric flask, dilute with methanol to volume, and mix.

**Standard preparation**—Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS in methanol to obtain a solution having a concentration of 50 µg per mL.

**Procedure**—Apply separate 10-µL portions of the *Standard preparation* and the *Test preparation* to the starting line of a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Dry the applied solutions with the aid of a stream of nitrogen. Develop the chromatogram, using as the solvent system a freshly prepared mixture of equal volumes of ether and ethyl acetate saturated with ammonium hydroxide, until the solvent front has moved about 13 cm from the origin. Remove the plate from the chamber, and air-dry for 30 minutes. Examine under short-wavelength UV light: the area and intensity of the only other spot in the test specimen chromatogram, other than the principal spot, are not greater than those of the spot from the *Standard preparation* (5.0%).

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 100 mg of chlorpromazine hydrochloride, to a 500-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Pipet 10 mL of the solution into a 250-mL separator, add about 20 mL of water, render alkaline with ammonium hydroxide, and extract with four 25-mL portions of ether. Extract the combined ether extracts with four 25-mL portions of 0.1 N hydrochloric acid, collecting the aqueous extracts in a 250-mL volumetric flask. Aerate to remove residual ether, add 0.1 N hydrochloric acid to volume, and mix. Dissolve a suitable quantity, accurately weighed, of USP Chlorpromazine Hydrochloride RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same acid to obtain a Standard solution having a known concentration of about 8 µg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 254 nm and at 277 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Calculate the quantity, in mg, of  $C_{17}H_{19}ClN_2S \cdot HCl$  in each mL of the Injection taken by the formula:

$$12.5C(A_{254} - A_{277})_U / V(A_{254} - A_{277})_S$$

in which C is the concentration, in µg per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution, V is the volume, in mL, of Injection taken, and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Injection (U) and the Standard solution (S), respectively.

## Chlorpromazine Hydrochloride Syrup

» Chlorpromazine Hydrochloride Syrup contains, in each 100 mL, not less than 190 mg and not more than 210 mg of  $C_{17}H_{19}ClN_2S \cdot HCl$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

**Identification**—

**A:** Transfer a volume of it, equivalent to about 20 mg of chlorpromazine hydrochloride, to a 125-mL separator. Add 10 mL of water, 2 mL of sodium hydroxide solution (1 in 2), and mix. Extract with three 30-mL portions of ether. Filter the combined ether extracts through anhydrous sodium sulfate. With the aid of a stream of nitrogen evaporate the ether to about 5 mL. Quantitatively transfer the solution to a 40-mL centrifuge tube. Evaporate with a stream of nitrogen and mild heat to dryness. Dissolve the residue in 100 mL of methanol to obtain the Test solution. Separately apply 15 µL of this Test solution and 15 µL of a Standard solution, containing 0.2 mg of USP Chlorpromazine Hydrochloride RS per mL of methanol, to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram in a chamber containing a freshly prepared mixture of ethyl acetate that has been saturated with ammonium hydroxide, ether, and methanol (75:25:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and spray with iodoplatinate reagent prepared by dissolving 100 mg of platinum chloride in 10 mL of 0.1 N hydrochloric acid, adding 25 mL of potassium iodide solution (1 in 25), 0.5 mL of formic acid, and diluting with water to 100 mL: the  $R_f$  value of the principal spot from the test solution corresponds to that obtained from the Standard solution.

**B:** Dilute a portion of the Syrup with an equal volume of water: the resulting solution responds to the tests for *Chloride* (191).

**Limit of chlorpromazine sulfoxide**—

**Chlorpromazine sulfoxide standard solution**—Transfer 5 mL of a solution in dilute hydrochloric acid (1 in 100) of USP Chlorpromazine Hydrochloride RS containing 10.6 mg per mL to a 50-mL volumetric flask. Add 2 mL of 30% hydrogen peroxide and heat at 60° for 10 minutes. Cool, dilute with 1 M sodium bisulfite to volume, and mix. Transfer 10.0 mL to a 60-mL separator, add 2 mL of sodium hydroxide solution (1 in 2), and mix. Extract with three 30-mL portions of ether. Filter the extracts through ether-wetted anhydrous sodium sulfate into a 250-mL conical flask. Cautiously evaporate the extracts to dryness. Dissolve the residue in 10.0 mL of methanol, and filter if necessary. Each mL of this solution contains 1 mg of chlorpromazine sulfoxide.

**Procedure**—Transfer an accurately measured volume of the Syrup, equivalent to about 20 mg of chlorpromazine hydrochloride, to a 125-mL separator. Add 10 mL of water and 2 mL of sodium hydroxide solution (1 in 2), and mix. Extract with three 30-mL portions of ether. Filter the combined ether extracts through anhydrous sodium sulfate. With the aid of a stream of nitrogen evaporate the ether to about 5 mL. Quantitatively transfer the solution to a 40-mL centrifuge tube. Evaporate with a stream of nitrogen and mild heat to dryness. Dissolve the residue in 1.0 mL of methanol to obtain the Test solution. Separately apply 15 µL of this Test solution and 15 µL of a *Chlorpromazine sulfoxide standard solution* to a thin-layer chromatographic plate (see



*Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Develop the chromatogram in a chamber containing a freshly prepared mixture of ethyl acetate that has been saturated with ammonium hydroxide, ether, and methanol (75:25:20) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, air-dry, and spray with lodoplatinate reagent prepared by dissolving 100 mg of platinum chloride in 10 mL of 0.1 N hydrochloric acid, adding 25 mL of potassium iodide solution (1 in 25) and 0.5 mL of formic acid, and diluting with water to 100 mL. The chromatogram from the Test solution may exhibit a secondary spot whose  $R_f$  value corresponds to, and whose size and intensity are not greater than, those of the spot from the *Chlorpromazine sulfoxide standard solution* (5.0%).

**Assay**—Transfer an accurately measured volume of Syrup, equivalent to about 10 mg of chlorpromazine hydrochloride, to a 50-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix. Proceed as directed in the Assay under *Chlorpromazine Hydrochloride Injection*, beginning with "Pipet 10 mL of the solution." Calculate the quantity, in mg, of  $C_{17}H_{19}ClN_2S \cdot HCl$  in each mL of the Syrup taken by the formula:

$$1.25C(A_{254} - A_{277})_U / V(A_{254} - A_{277})_S$$

in which C is the concentration, in  $\mu g$  per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution; V is the volume, in mL, of Syrup taken; and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Syrup (U) and the Standard solution (S), respectively.

### Chlorpromazine Hydrochloride Tablets

» Chlorpromazine Hydrochloride Tablets contain not less than 95.0 percent and not more than 105.0 percent of the labeled amount of  $C_{17}H_{19}ClN_2S \cdot HCl$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

#### USP Reference standards (11)—

USP Chlorpromazine Hydrochloride RS

[NOTE—Throughout the following procedures, protect test or assay specimens, the Reference Standard, and solutions containing them by conducting the procedures without delay, under subdued light, or using low-actinic glassware.]

#### Identification—

A: Tablets respond to Identification test B under *Chlorpromazine Hydrochloride*.

B: Digest a quantity of powdered Tablets, equivalent to about 25 mg of chlorpromazine hydrochloride, with 25 mL of water, and filter: the solution so obtained responds to Identification test C under *Chlorpromazine Hydrochloride*.

#### Dissolution (711)—

Medium: 0.1 N hydrochloric acid; 900 mL.

Apparatus 1: 50 rpm.

Time: 30 minutes.

**Procedure**—Determine the amount of  $C_{17}H_{19}ClN_2S \cdot HCl$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 254 nm of filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, in comparison with a Standard solution having a known concentration of USP Chlorpromazine Hydrochloride RS in the same medium.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{17}H_{19}ClN_2S \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

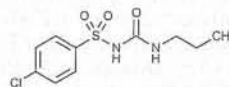
**Other alkylated phenothiazines**—Transfer a portion of finely powdered Tablets, equivalent to 50 mg of chlorpromazine hydrochloride, to a stoppered centrifuge tube, add 10 mL of methanol, shake vigorously, and centrifuge (remove any sugar coating by prior washing with water). Proceed as directed in the test for *Other alkylated phenothiazines* under *Chlorpromazine*, beginning with "Dissolve a suitable quantity of USP Chlorpromazine Hydrochloride RS." The area and intensity of any spot, other than the principal spot, from the solution from the Tablets are not greater than those of the spot from the *Diluted standard solution* (0.5%).

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of chlorpromazine hydrochloride, to a 500-mL volumetric flask. Add about 200 mL of water and 5 mL of hydrochloric acid, insert the stopper, and shake for about 10 minutes. Dilute with water to volume, and mix. Filter a portion of the solution, discarding the first 50 mL of the filtrate. Treat 10.0 mL of the filtrate as directed in the Assay under *Chlorpromazine Hydrochloride Injection*, beginning with "Pipet 10 mL of the solution." Calculate the quantity, in mg, of  $C_{17}H_{19}ClN_2S \cdot HCl$  in the portion of Tablets taken by the formula:

$$12.5C(A_{254} - A_{277})_U / (A_{254} - A_{277})_S$$

in which C is the concentration, in  $\mu g$  per mL, of USP Chlorpromazine Hydrochloride RS in the Standard solution, and the parenthetical expressions are the differences in the absorbances of the two solutions at the wavelengths indicated by the subscripts, for the solution from the Tablets (U) and the Standard solution (S), respectively.

### Chlorpropamide



$C_{10}H_{13}ClN_2O_3S$  276.74

Benzenesulfonamide, 4-chloro-N-[(propylamino)carbonyl]-1-[(p-chlorophenyl)sulfonyl]-3-propylurea [94-20-2].

» Chlorpropamide contains not less than 97.0 percent and not more than 103.0 percent of  $C_{10}H_{13}ClN_2O_3S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

#### USP Reference standards (11)—

USP Chlorpropamide RS

#### Identification—

A: Infrared Absorption (197K).

B: It responds to the *Thin-Layer Chromatographic Identification Test* (201). Prepare the test solution by dissolving an accurately weighed quantity of Chlorpropamide in acetone to obtain a solution containing 1 mg per mL. Develop the chromatogram in a solvent system consisting of a mixture of methylene chloride, methanol, cyclohexane, and ammonium hydroxide (100:50:30:10).

**Melting range** (741): between 126° and 129°.

**Loss on drying** (731)—Dry it in vacuum at 60° for 2 hours: it loses not more than 1.0% of its weight.



**Residue on ignition** (281): not more than 0.4%.

**Selenium** (291): 0.003%.

**Delete the following:**

• **Heavy metals, Method II** (231): 0.003%. (Official 1-Jan-2018)

**Assay—**

**Mobile phase**—Prepare a filtered and degassed mixture of equal volumes of acetonitrile and dilute glacial acetic acid (1 in 100). [NOTE—Do not exceed 50% of acetonitrile.] Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorpropamide RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.05 mg per mL.

**Assay preparation**—Transfer about 50 mg of Chlorpropamide, accurately weighed, to a 100-mL volumetric flask, add *Mobile phase* to volume, and mix. Transfer 10 mL of this solution to a second 100-mL volumetric flask, add *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 240-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the tailing factor for the analyte peak is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{10}H_{13}ClN_2O_3S$  in the portion of Chlorpropamide taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpropamide RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorpropamide Tablets

» Chlorpropamide Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{10}H_{13}ClN_2O_3S$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Chlorpropamide RS

**Identification**—Shake a quantity of finely powdered Tablets, equivalent to about 100 mg of chlorpropamide, with 20 mL of 1 N hydrochloric acid, and extract with 50 mL of chloroform. Filter the chloroform through chloroform-washed cotton into a suitable beaker, and evaporate the chloroform on a steam bath with the aid of a current of dry air to dryness. Dry the residue at 105° for 1 hour; the residue so obtained responds to the *Identification* tests under *Chlorpropamide*.

**Dissolution** (711)—

**Medium:** water; 900 mL.

**Apparatus 2:** 50 rpm.

**Time:** 60 minutes.

**Procedure**—Determine the amount of  $C_{10}H_{13}ClN_2O_3S$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 230 nm of filtered portions of the solution under test, suitably diluted with 0.1 N hydrochloric acid in comparison with a Standard solution having a known concentration of USP Chlorpropamide RS in 0.1 N hydrochloric acid. [NOTE—A volume of alcohol not exceeding 10% of the final volume of the Standard solution may be used to dissolve the USP Chlorpropamide RS.]

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{10}H_{13}ClN_2O_3S$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay—**

**Mobile phase, Standard preparation, and Chromatographic system**—Proceed as directed in the *Assay* under *Chlorpropamide*.

**Assay preparation**—Weigh and finely powder not less than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 50 mg of chlorpropamide, to a 100-mL volumetric flask. Add *Mobile phase* to volume, mix, and filter, discarding the first 10 mL of the filtrate. Pipet 10 mL of the filtrate into a second 100-mL volumetric flask, add *Mobile phase* to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay* under *Chlorpropamide*. Calculate the quantity, in mg, of  $C_{10}H_{13}ClN_2O_3S$  in the portion of Tablets taken by the formula:

$$1000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Chlorpropamide RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlortetracycline Bisulfate

» Chlortetracycline Bisulfate has a potency equivalent to not less than 760 µg of chlortetracycline hydrochloride ( $C_{22}H_{23}ClN_2O_8 \cdot HCl$ ) per mg, calculated on the dried and butyl alcohol-free basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate that it is intended for veterinary use only.

**USP Reference standards** (11)—

USP Chlortetracycline Hydrochloride RS

**Identification, Ultraviolet Absorption** (197U)—

**Solution:** 40 µg per mL.

**Medium:** 0.1 N hydrochloric acid.

**Absorptivity** at 368 nm, calculated on the dried and butyl alcohol-free basis, is not less than 83.0% and not more than 95.0% of that of the USP Chlortetracycline Hydrochloride RS, the potency of the Reference Standard being taken into account.

**Crystallinity** (695): meets the requirements.

**Loss on drying** (731)—Dry it in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

**Sulfate content**—Transfer about 1 g, accurately weighed, to a 250-mL beaker, and dissolve in about 100 mL of water.



Neutralize the solution with 7.5 N ammonium hydroxide to litmus paper, and warm. Filter, and wash the filter with warm water. Neutralize the filtrate with 6 N hydrochloric acid to litmus, and add an additional 4 mL of 6 N hydrochloric acid. Heat the solution to boiling, and add, with constant stirring, sufficient boiling barium chloride TS to precipitate all of the sulfate. Add an additional 2 mL of barium chloride TS, and digest on a steam bath for 1 hour. Filter the mixture through ashless filter paper, transferring the residue quantitatively to the filter, and wash the residue with hot water until no precipitate is obtained when 1 mL of silver nitrate TS is added to 5 mL of washing. Transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents to constant weight. Perform a blank determination concurrently with the test specimen determination, and subtract the weight of residue obtained from that obtained in the test specimen determination to obtain the weight of residue attributable to the sulfate content of the specimen: not less than 15.0% is found, calculated on the dried and butyl alcohol-free basis.

#### Butyl alcohol—

**Ceric ammonium nitrate solution**—Dissolve 20 g of ceric ammonium nitrate in 4 N nitric acid to obtain 100 mL of solution.

**Standard preparations**—Transfer about 3 g of butyl alcohol, accurately weighed, to a 1000-mL volumetric flask containing 800 mL of water, shake to dissolve, dilute with water to volume, and mix (*Standard preparation 1*). Transfer 10.0 mL of *Standard preparation 1* and 1 drop of dimethicone to a 50-mL distilling flask equipped with a condenser and an extension that reaches into a collecting tube maintained in an ice-water bath. Distill slowly, and collect about 8 mL of distillate. Warm the distillate to ambient temperature, and transfer with the aid of water to a 10-mL volumetric flask. Dilute with water to volume, and mix (*Standard preparation 2*).

**Test preparation**—Transfer an accurately weighed specimen, equivalent to about 30 mg of butyl alcohol, to a 50-mL distilling flask equipped with a condenser and an extension that reaches into a collecting tube maintained in an ice bath. Add 25 mL of water and 1 drop of dimethicone to the distilling flask. Distill slowly, and collect about 8 mL of the distillate. Warm the distillate to ambient temperature, and transfer with the aid of water to a 10-mL volumetric flask. Dilute with water to volume, and mix.

**Procedure**—To four separate test tubes add, respectively, 5.0 mL of *Standard preparation 1*, 5.0 mL of *Standard preparation 2*, 5.0 mL of *Test preparation*, and 5.0 mL of water to provide a blank. To each add 2.0 mL of *Ceric ammonium nitrate solution*, and mix. Concomitantly determine the absorbances of the solutions from the *Standard preparations* and the *Test preparation* at the wavelength of maximum absorbance at about 475 nm, with a suitable spectrophotometer, using the blank to set the instrument to zero. In a suitable determination, the absorbance of the solution from *Standard preparation 2* is not less than 98.0% of the absorbance of the solution from *Standard preparation 1*. Calculate the percentage of butyl alcohol in the specimen taken by the formula:

$$1000(W_s / W_u)(A_u / A_s)$$

in which  $W_s$  is the weight, in g, of butyl alcohol taken to prepare *Standard preparation 1*,  $W_u$  is the weight, in mg, of specimen taken, and  $A_u$  and  $A_s$  are the absorbances of the solutions from the *Test preparation* and *Standard preparation 2*, respectively: not more than 15.0% is found.

**Assay**—Proceed with Chlortetracycline Bisulfate as directed for chlortetracycline under *Antibiotics—Microbial Assays* (81).

## Chlortetracycline and Sulfamethazine Bisulfates Soluble Powder

» Chlortetracycline and Sulfamethazine Bisulfates Soluble Powder is a dry mixture of Chlortetracycline Bisulfate and Sulfamethazine Bisulfate and one or more suitable buffers and diluents. It contains the equivalent of not less than 85.0 percent and not more than 125.0 percent of the labeled amounts of chlortetracycline hydrochloride ( $C_{22}H_{23}ClN_2O_8 \cdot HCl$ ) and sulfamethazine ( $C_{12}H_{14}N_4O_2S$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Label it to indicate that it is intended for veterinary use only.

#### USP Reference standards (11)—

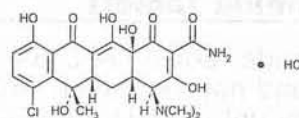
USP Chlortetracycline Hydrochloride RS

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

**Assay for chlortetracycline hydrochloride**—Proceed as directed for chlortetracycline under *Antibiotics—Microbial Assays* (81), using an accurately weighed quantity of Powder, equivalent to about 100 mg of chlortetracycline hydrochloride, dissolved in an accurately measured volume of 0.01 N hydrochloric acid to obtain a stock solution having a convenient concentration. Dilute an accurately measured volume of this stock solution quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

**Assay for sulfamethazine**—Proceed as directed under *Nitrite Titration* (451), using an accurately weighed quantity of Powder, equivalent to about 500 mg of sulfamethazine. Each mL of 0.1 M sodium nitrite is equivalent to 27.83 mg of sulfamethazine ( $C_{12}H_{14}N_4O_2S$ ).

## Chlortetracycline Hydrochloride



$C_{22}H_{23}ClN_2O_8 \cdot HCl$  515.34

2-Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride [4S-(4 $\alpha$ ,4a $\alpha$ ,5a $\alpha$ ,6 $\beta$ ,12a $\alpha$ )]-

7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [64-72-2].

» Chlortetracycline Hydrochloride has a potency of not less than 900  $\mu$ g of  $C_{22}H_{23}ClN_2O_8 \cdot HCl$  per mg.

NOTE—Chlortetracycline Hydrochloride labeled solely for use in preparing oral veterinary dosage forms has a potency of not less than 820  $\mu$ g of  $C_{22}H_{23}ClN_2O_8 \cdot HCl$  per mg.



**Packaging and storage**—Preserve in tight, light-resistant containers.

**Labeling**—Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.

**USP Reference standards** (11)—

USP Chlortetracycline Hydrochloride RS

USP Oxytetracycline RS

USP Tetracycline Hydrochloride RS

**Identification**—

**A:** Proceed as directed for *Method II* under *Identification—Tetracyclines* (193), using a methanol solution containing 0.5 mg per mL as the *Test solution* and a methanol solution containing in each mL 0.5 mg of USP Chlortetracycline Hydrochloride RS, 0.5 mg of USP Oxytetracycline RS, and 0.5 mg of USP Tetracycline Hydrochloride RS as the *Resolution solution*.

**B:** A solution (1 in 20) meets the requirements of the tests for *Chloride* (191).

**Specific rotation** (781S): between  $-235^{\circ}$  and  $-250^{\circ}$ .

*Test solution:* 5 mg per mL, in water, that has been allowed to stand in the dark for 30 minutes.

**Crystallinity** (695): meets the requirements.

**Sterility Tests** (71)—Where the label states that Chlortetracycline Hydrochloride is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 6 g of specimen aseptically dissolved in 200 mL of *Fluid D* being used.

**pH** (791): between 2.3 and 3.3, in a solution containing 10 mg per mL.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at  $60^{\circ}$  for 3 hours: it loses not more than 2.0% of its weight.

**Assay**—Proceed with Chlortetracycline Hydrochloride as directed under *Antibiotics—Microbial Assays* (81).

## Chlortetracycline Hydrochloride Ointment

» Chlortetracycline Hydrochloride Ointment contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of  $C_{22}H_{23}ClN_2O_8 \cdot HCl$  in a suitable ointment base.

**Packaging and storage**—Preserve in collapsible tubes or in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Chlortetracycline Hydrochloride RS

**Minimum fill** (755): meets the requirements.

**Water Determination, Method I** (921): not more than 0.5%, 20 mL of a mixture of toluene and methanol (7:3) being used in place of methanol in the titration vessel.

**Assay**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using an accurately weighed quantity of Ointment, equivalent to about 30 mg of chlortetracycline hydrochloride, shaken in a separator with about 50 mL of ether, and extracted with four 20-mL portions of 0.01 N hydrochloric acid. Combine the aqueous extracts in a 100-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Dilute this stock solution quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the medium dose level of the Standard.

## Chlortetracycline Hydrochloride Ophthalmic Ointment

**DEFINITION**

Chlortetracycline Hydrochloride Ophthalmic Ointment contains NLT 90.0% and NMT 125.0% of the labeled amount of chlortetracycline hydrochloride ( $C_{22}H_{23}ClN_2O_8 \cdot HCl$ ).

**ASSAY**

• **PROCEDURE**

(See *Antibiotics—Microbial Assays* (81).)

**Sample solution:** Shake a portion of Ophthalmic Ointment containing nominally 10 mg of chlortetracycline hydrochloride in a separator with 50 mL of ether, and extract with four 20-mL portions of 0.01 N hydrochloric acid. Combine the aqueous extracts in a 100-mL volumetric flask, and dilute with 0.01 N hydrochloric acid to volume.

**Analysis:** Proceed as directed in the chapter. Dilute the *Sample solution* with water to obtain a *Test Dilution* having a chlortetracycline hydrochloride concentration that is nominally equivalent to the median level of the standard.

**Acceptance criteria:** 90.0%–125.0%

**SPECIFIC TESTS**

- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes.
- **USP REFERENCE STANDARDS** (11)  
USP Chlortetracycline Hydrochloride RS

## Chlortetracycline Hydrochloride Soluble Powder

» Chlortetracycline Hydrochloride Soluble Powder contains not less than 90.0 percent and not more than 125.0 percent of the labeled amount of  $C_{22}H_{23}ClN_2O_8 \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers, protected from light.

**Labeling**—Label it to indicate that it is intended for oral veterinary use only.

**USP Reference standards** (11)—

USP Chlortetracycline Hydrochloride RS

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at  $60^{\circ}$  for 3 hours: it loses not more than 2.0% of its weight.

**Assay**—

*Assay preparation 1* (where it is labeled on a weight basis)—Dissolve about 3 g of Powder in an accurately measured volume of 0.01 N hydrochloric acid sufficient to obtain a solution containing not less than 1000  $\mu$ g of chlortetracycline hydrochloride ( $C_{22}H_{23}ClN_2O_8 \cdot HCl$ ) per mL.

*Assay preparation 2* (where the label states the amount of chlortetracycline in the immediate container)—Transfer the contents of 1 container of Powder to an accurately measured volume of 0.01 N hydrochloric acid sufficient to obtain a solution containing not less than 1000  $\mu$ g of chlortetracycline hydrochloride ( $C_{22}H_{23}ClN_2O_8 \cdot HCl$ ) per mL.



**Procedure**—Proceed with Powder as directed for chlortetracycline under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively and stepwise with water to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Chlortetracycline Hydrochloride Tablets

» Chlortetracycline Hydrochloride Tablets contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{22}H_{23}ClN_2O_8 \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers, protected from light.

**Labeling**—Label the Tablets to indicate that they are intended for veterinary use only.

### USP Reference standards (11)—

USP Chlortetracycline Hydrochloride RS

USP Oxytetracycline RS

USP Tetracycline Hydrochloride RS

**Identification**—Shake a suitable quantity of finely ground Tablet powder with methanol to obtain a solution containing about 0.5 mg of chlortetracycline hydrochloride per mL, and filter. Using the filtrate so obtained as the *Test solution*, and a methanol solution containing in each mL 0.5 mg of USP Chlortetracycline Hydrochloride RS, 0.5 mg of USP Oxytetracycline RS, and 0.5 mg of USP Tetracycline Hydrochloride RS as the *Resolution solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

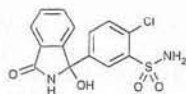
**Disintegration** (701): 1 hour, simulated gastric fluid TS being used as the test medium in place of water.

**Uniformity of dosage units** (905): meet the requirements for *Weight Variation*.

**Water Determination, Method I** (921): not more than 3.0%, or where the Tablets have a diameter of greater than 15 mm, not more than 6.0%, a quantity of finely ground Tablet powder, accurately weighed, being used.

**Assay**—Transfer not less than 5 Tablets to a high-speed glass blender jar containing an accurately measured volume of 0.01 N hydrochloric acid, so that after blending for 3 to 5 minutes a solution of convenient concentration is obtained. Proceed as directed for chlortetracycline under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of this solution diluted quantitatively and stepwise with water to obtain a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Chlorthalidone



$C_{14}H_{11}ClN_2O_4S$  338.77

Benzenesulfonamide, 2-chloro-5-(2,3-dihydro-1-hydroxy-3-oxo-1H-indol-1-yl)-

2-Chloro-5-(1-hydroxy-3-oxo-1-indolyl)benzenesulfonamide [77-36-1].

» Chlorthalidone contains not less than 98.0 percent and not more than 102.0 percent of  $C_{14}H_{11}ClN_2O_4S$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

### USP Reference standards (11)—

USP Chlorthalidone RS

USP Chlorthalidone Related Compound A RS

4'-Chloro-3'-sulfamoyl-2-benzophenone carboxylic acid.

### Identification—

**A: Infrared Absorption** (197M).

**B: Ultraviolet Absorption** (197U)—

*Solution:* 100 µg per mL.

*Medium:* 2 N hydrochloric acid in methanol (1 in 50).

Absorptivities at 275 nm, calculated on the dried basis, do not differ by more than 4.0%.

**C:** Dissolve about 50 mg in 3 mL of sulfuric acid: an intense yellow color develops.

**Loss on drying** (731)—Dry about 2 g, accurately weighed, at 105° for 4 hours: it loses not more than 0.4% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chloride** (221)—Shake 1.0 g with 40 mL of water for 5 minutes, and filter through chloride-free filter paper previously rinsed with water: the filtrate shows no more chloride than corresponds to 0.50 mL of 0.020 N hydrochloric acid (0.035%).

### Delete the following:

• **Heavy metals, Method II** (231): 0.001%. • (Official 1-Jan-2018)

**Limit of chlorthalidone related compound A**—Proceed as directed in the *Assay*, except to calculate the percentage of chlorthalidone related compound A in the portion of Chlorthalidone taken by the formula:

$$0.1(C_R / C_T)(R_U / R_S)$$

in which  $C_R$  is the concentration, in µg per mL, of USP Chlorthalidone Related Compound A RS in the *Standard preparation*;  $C_T$  is the concentration, in mg per mL, of Chlorthalidone in the *Assay preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of chlorthalidone related compound A to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively: not more than 1.0% is present. [NOTE—USP Chlorthalidone Related Compound A RS is 4'-chloro-3'-sulfamoyl-2-benzophenone carboxylic acid (CCA).]

### Assay—

**Mobile phase**—Prepare a suitable degassed mixture of 0.01 M dibasic ammonium phosphate and methanol (3:2), adjust dropwise with phosphoric acid to a pH of  $5.5 \pm 0.1$ , and filter.

**Internal standard solution**—Prepare a solution of 2,7-naphthalenediol in methanol having a concentration of about 1.0 mg per mL.

**Chlorthalidone related compound A solution**—Prepare a solution of USP Chlorthalidone Related Compound A RS in methanol having a known concentration of about 5 µg per mL.

**Standard preparation**—Prepare a solution of USP Chlorthalidone RS in methanol having a known concentration of about 1 mg per mL. Pipet 5 mL of this solution into a 50-mL volumetric flask containing 5.0 mL of *Internal standard solution* and 10.0 mL of *Chlorthalidone related compound A solution*. Dilute with water to volume, and mix. This solution contains about 0.1 mg of chlorthalidone and about 1 µg of chlorthalidone related compound A per mL.



**Assay preparation**—Transfer about 50 mg of Chlorthalidone, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with methanol to volume, and mix. Pipet 5 mL of this solution into a 50-mL volumetric flask containing 5.0 mL of *Internal standard solution* and 10.0 mL of methanol. Dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for chlorthalidone related compound A, 0.8 for chlorthalidone, and 1.0 for the internal standard; the resolution, *R*, between chlorthalidone and chlorthalidone related compound A, and between chlorthalidone and the internal standard is not less than 1.5; the tailing factor for chlorthalidone and chlorthalidone related compound A is not more than 2.0; and the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{14}H_{11}ClN_2O_4S$  in the portion of Chlorthalidone taken by the formula:

$$500C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Chlorthalidone RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of chlorthalidone to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorthalidone Tablets

» Chlorthalidone Tablets contain not less than 92.0 percent and not more than 108.0 percent of the labeled amount of  $C_{14}H_{11}ClN_2O_4S$ .

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Chlorthalidone RS

**Identification**—

**A:** Digest a quantity of powdered Tablets, equivalent to about 100 mg of chlorthalidone, with 10 mL of acetone on a steam bath for about 5 minutes. Filter the solution into a 50-mL beaker, add 20 mL of water, and boil on the steam bath for about 5 minutes, passing a gentle current of air above the solution to remove the acetone. Cool in an ice bath, filter, and dry the crystals at 105° for 4 hours: the crystals so obtained respond to *Identification test A* under *Chlorthalidone*.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay*.

**Dissolution** (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 60 minutes.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorthalidone RS in methanol to obtain a solution having a known concentration of about 5 mg per mL.

**Procedure**—Determine the amount of  $C_{14}H_{11}ClN_2O_4S$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 275 nm of filtered portions of the solution under test, suitably diluted with water, in comparison with a quantitative dilution in water of the *Standard preparation* having a known concentration of USP Chlorthalidone RS comparable to the concentration of the solution under test.

**Tolerances**—Not less than 70% (*Q*) of the labeled amount of  $C_{14}H_{11}ClN_2O_4S$  is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Mobile phase and Internal standard solution**—Prepare as directed in the *Assay* under *Chlorthalidone*.

**Standard preparation**—Prepare as directed in the *Assay* under *Chlorthalidone*, except to substitute 10.0 mL of methanol for the *CCA solution*.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of Chlorthalidone, to a 100-mL volumetric flask. Dissolve in about 50 mL of methanol, shake for 30 minutes, dilute with methanol to volume, and mix. Transfer about 30 mL of this solution to a 50-mL centrifuge tube, and centrifuge for 10 minutes. Pipet 5 mL of the clear supernatant into a 50-mL volumetric flask containing 5.0 mL of *Internal standard solution* and 10.0 mL of methanol. Dilute with water to volume, and mix.

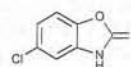
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1.0 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation is not more than 2.0%, and the resolution factor between chlorthalidone and the internal standard is not less than 1.5. The tailing factors for the chlorthalidone and internal standard peaks are not more than 2.0.

**Procedure**—Separately inject equal volumes (about 25 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.8 for chlorthalidone and 1.0 for the internal standard. Calculate the quantity, in mg, of  $C_{14}H_{11}ClN_2O_4S$  in the portion of Tablets taken by the formula:

$$1000C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Chlorthalidone RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of chlorthalidone to the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Chlorzoxazone



$C_7H_4ClNO_2$  169.57

2(3*H*)-Benzoxazolone, 5-chloro-

5-Chloro-2-benzoxazolinone [95-25-0].

» Chlorzoxazone contains not less than 98.0 percent and not more than 102.0 percent of  $C_7H_4ClNO_2$ , calculated on the dried basis.



**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Chlorzoxazone RS

USP Chlorzoxazone Related Compound A RS

2-Amino-4-chlorophenol.

C<sub>6</sub>H<sub>6</sub>ClNO 143.57

**Identification**—

A: Infrared Absorption (197K): previously dried.

B: Ultraviolet Absorption (197U)—

Solution: 20 µg per mL.

Medium: methanol.

**Melting range** (741): between 189° and 194°.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

**Delete the following:**

• **Heavy metals, Method II** (231): 0.002%. (Official 1-Jan-2018)

**Residue on ignition** (281): not more than 0.15%.

**Chromatographic purity**—Prepare a *Test solution* in methanol containing 20 mg per mL. Dissolve a suitable quantity of USP Chlorzoxazone Related Compound A RS (2-Amino-4-chlorophenol) in methanol to obtain a solution containing 100 µg per mL (*Standard solution A*). Dissolve a suitable quantity of *p*-chlorophenol in methanol to obtain a solution containing 50 µg per mL (*Standard solution B*). Apply separate 10 µL portions of the three solutions to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of hexane and dioxane (63:37) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by examination under short-wavelength UV light: any spot obtained from the *Test solution*, other than one corresponding to chlorzoxazone, does not exceed, in size or intensity, the principal spot obtained from *Standard solution A*, corresponding to not more than 0.5% of any individual impurity. Expose the plate to iodine vapors in a closed chamber, and locate the spots: any spot obtained from the *Test solution*, other than one corresponding to chlorzoxazone, does not exceed, in size or intensity, the principal spot obtained from *Standard solution B*, corresponding to not more than 0.25% of any individual impurity.

**Chlorine content**—Dissolve about 300 mg, accurately weighed, in 10 mL of alcohol in a suitable flask. Add 3.5 g of Raney's nickel-aluminum catalyst, and connect to a suitable reflux condenser. Chill the flask in an ice bath, and add through the condenser 75 mL of 2.5 N sodium hydroxide. When the reaction has subsided, remove the ice bath. After 10 minutes, heat the flask gently, gradually increasing the heat until the mixture refluxes rapidly. After 90 minutes from the time of the addition of the alkali, discontinue heating, cool, and rinse the condenser with water, collecting the rinsings in the flask. Transfer the liquid to a 200-mL volumetric flask, wash the residue with water, and add the washing to the volumetric flask. Dilute with water to volume, and mix. Transfer 100.0 mL of this solution to a beaker, neutralize, then acidify (using congo red as the indicator) by adding nitric acid dropwise with mixing. Titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, using silver and calomel electrodes. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl: the content of Cl, calculated on the dried basis, is between 20.6% and 21.2%.

**Assay**—Transfer about 50 mg of Chlorzoxazone, accurately weighed, to a 100-mL volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix. Transfer

4.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix. Concomitantly determine the absorbances of this solution and a Standard solution of USP Chlorzoxazone RS in methanol at a concentration of about 20 µg per mL in 1-cm cells at the wavelength of maximum absorbance at about 282 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of C<sub>7</sub>H<sub>4</sub>ClNO<sub>2</sub> in the Chlorzoxazone taken by the formula:

$$2.5C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Chlorzoxazone RS in the Standard solution; and A<sub>U</sub> and A<sub>S</sub> are the absorbances of the solution of Chlorzoxazone and the Standard solution, respectively.

## Chlorzoxazone Tablets

» Chlorzoxazone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>7</sub>H<sub>4</sub>ClNO<sub>2</sub>.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Chlorzoxazone RS

**Identification**—

A: Disperse a portion of powdered Tablets, equivalent to about 100 mg of chlorzoxazone, in 100 mL of methanol, shake for 15 minutes, and filter. Transfer 2.0 mL of the filtrate to a 100-mL volumetric flask, dilute with methanol to volume, and mix: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Chlorzoxazone RS, concomitantly measured.

B: The chromatogram of the *Assay preparation* obtained in the *Assay* exhibits a major peak for chlorzoxazone, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation* obtained in the *Assay*.

**Dissolution** (711)—[NOTE—Use 2-liter vessels for this test.]

Medium: pH 6.8 phosphate buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*); 1800 mL.

Apparatus 2: 75 rpm.

Time: 60 minutes.

**Procedure**—Determine the amount of C<sub>7</sub>H<sub>4</sub>ClNO<sub>2</sub> dissolved by employing UV absorption at the wavelength of maximum absorbance at about 284 nm on filtered portions of the solution under test, diluted with *Medium*, if necessary, in comparison with a Standard solution of USP Chlorzoxazone RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of C<sub>7</sub>H<sub>4</sub>ClNO<sub>2</sub> is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**1% Acetic acid solution**—Dilute 10 mL of glacial acetic acid with water to make 1000 mL of solution.

**Mobile phase**—Prepare a filtered and degassed mixture of water, acetonitrile, and glacial acetic acid (70:30:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of phenacetin in acetonitrile containing about 1.25 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Chlorzoxazone RS quantitatively in *Mobile*



*phase* to obtain a stock solution having a known concentration of about 1.25 mg per mL. Transfer 5.0 mL of this stock solution to a 50-mL volumetric flask containing 10.0 mL of *Internal standard solution*, dilute with 1% *Acetic acid solution* to volume, and mix.

*Resolution solution*—Prepare a solution of *p*-chlorophenol in acetonitrile containing about 8.5 mg per mL. Transfer 1 mL of this solution to a 50-mL volumetric flask containing 4 mL of the stock solution used to prepare the *Standard preparation* and 10 mL of *Internal standard solution*, dilute with 1% *Acetic acid solution* to volume, and mix.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 125 mg of chlorzoxazone, to a 100-mL volumetric flask, add about 70 mL of acetonitrile, and shake by mechanical means for about 30 minutes. Dilute with acetonitrile to volume, and mix. Filter a portion of this solution, discarding the first 10 mL of the filtrate. Transfer 5.0 mL of the clear filtrate to a 50-mL volumetric flask containing 10.0 mL of *Internal standard solution*, dilute with 1% *Acetic acid solution* to volume, and mix.

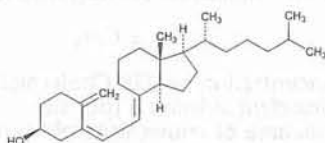
*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 280-nm detector and a 4-mm × 30-cm column containing packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.7 for phenacetin, 1.0 for chlorzoxazone, and 1.2 for *p*-chlorophenol; and the resolution, *R*, between the chlorzoxazone peak and the *p*-chlorophenol peak is not less than 2.0. Chromatograph the *Standard preparation*, and record the responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the area responses for the major peaks. Calculate the quantity, in mg, of C<sub>27</sub>H<sub>44</sub>O<sub>2</sub> in the portion of Tablets taken by the formula:

$$1000C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Chlorzoxazone RS in the *Standard preparation*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak response ratios of the chlorzoxazone peak to the phenacetin peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cholecalciferol



C<sub>27</sub>H<sub>44</sub>O  
9,10-Secocholesta-5,7,10(19)-trien-3-ol, (3β,5Z,7E)-;  
Cholecalciferol [67-97-0]. 384.64

### DEFINITION

Cholecalciferol contains NLT 97.0% and NMT 103.0% of cholecalciferol (C<sub>27</sub>H<sub>44</sub>O).

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)  
Wavelength range: 2–12 µm
- **B. ULTRAVIOLET ABSORPTION** (197U)  
Analytical wavelength: 265 nm  
Sample solution: 10 µg/mL in alcohol  
Acceptance criteria: Meets the requirements in the chapter. Absorptivities do not differ by more than 3.0%.
- **C.**  
Sample solution: 0.5 mg in 5 mL of chloroform  
Analysis: Add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid to the *Sample solution*, and shake vigorously.  
Acceptance criteria: A bright red color is produced, and it rapidly changes through violet and blue to green.
- **D. THIN-LAYER CHROMATOGRAPHY**  
[NOTE—For the *Standard solution* and the *Sample solution*, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and use the solutions immediately.]  
Diluent: 10 mg/mL of squalane in chloroform  
Standard solution: 50 mg/mL of USP Cholecalciferol RS in Diluent  
Sample solution: 50 mg/mL of Cholecalciferol in Diluent  
Chromatographic system  
(See *Chromatography* <621>, *Thin-Layer Chromatography*.)  
Mode: TLC  
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture  
Application volume: 10 µL  
Developing solvent system: Cyclohexane and diethyl ether (1:1)  
Spray reagent: 20 mg/mL of acetyl chloride in antimony trichloride TS  
Analysis  
Samples: *Standard solution* and *Sample solution*  
[NOTE—Perform the development and subsequent operations in the dark.]  
Place the plate in a chamber containing and equilibrated with *Developing solvent system*. Develop until the solvent front has moved about 15 cm above the line of application. Remove the plate, allow the solvent to evaporate, and spray with *Spray reagent*.  
Acceptance criteria: The *Sample solution* shows a yellowish-orange area (cholecalciferol) having the same *R<sub>f</sub>* value as the area of the *Standard solution* and may show below the cholecalciferol area a violet area, attributed to 7-dehydrocholesterol.

### ASSAY

#### PROCEDURE

**Dehydrated hexane:** Prepare a chromatographic column by packing a chromatographic tube, 8 cm × 60 cm, with 500 g of 50- to 250-µm chromatographic siliceous earth, activated by drying at 150° for 4 h. (See *Chromatography* <621>, *Column Chromatography*.) Pass 500 mL of hexane through the column, and collect the eluate in a glass-stoppered flask.

**Mobile phase:** *n*-Amyl alcohol in *Dehydrated hexane* (3 in 1000)

**System suitability solution:** 250 mg of USP Vitamin D Assay System Suitability RS in 10 mL of a mixture of toluene and *Mobile phase* (1:1). Heat this solution, under reflux, at 90° for 45 min, and cool. [NOTE—This solution contains cholecalciferol, precholecalciferol, and *trans*-cholecalciferol.]

[NOTE—For the stock solutions, follow these procedures: use low-actinic glassware, dissolve the samples without heating, and prepare the solutions fresh daily.]

**Standard stock solution:** 0.6 mg/mL of USP Cholecalciferol RS in toluene



**Standard solution:** 120 µg/mL of USP Cholecalciferol RS in *Mobile phase*, prepared from *Standard stock solution*

**Sample stock solution:** 0.6 mg/mL of Cholecalciferol in toluene

**Sample solution:** 120 µg/mL of Cholecalciferol in *Mobile phase*, prepared from *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L3

**Injection size:** 5–10 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for precholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.4, 0.5, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.0 between *trans*-cholecalciferol and precholecalciferol

**Relative standard deviation:** NMT 2.0% for the peak response of cholecalciferol

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cholecalciferol (C<sub>27</sub>H<sub>44</sub>O) in the portion of Cholecalciferol taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Cholecalciferol RS in the *Standard solution* (µg/mL)

$C_u$  = concentration of Cholecalciferol in the *Sample solution* (µg/mL)

**Acceptance criteria:** 97.0%–103.0%

#### SPECIFIC TESTS

##### • OPTICAL ROTATION, *Specific Rotation* (781S)

**Sample solution:** 5 mg/mL in alcohol. [NOTE—Prepare and use the solution without delay. Use Cholecalciferol from a container opened not longer than 30 min.]

**Acceptance criteria:** +105° to +112°

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in hermetically sealed containers under nitrogen, and store in a cool place protected from light.

##### • USP REFERENCE STANDARDS (11)

USP Cholecalciferol RS

USP Vitamin D Assay System Suitability RS

## Cholecalciferol Capsules

#### DEFINITION

Cholecalciferol Capsules contain a solution of Cholecalciferol in an edible oil or other suitable vehicle. Cholecalciferol Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of vitamin D as cholecalciferol (C<sub>27</sub>H<sub>44</sub>O).

#### IDENTIFICATION

- **A.** The retention time of the major peak for cholecalciferol of the *Sample solution* corresponds to that of *Standard solution A*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

[NOTE—Use low-actinic glassware throughout this procedure.]

**Mobile phase:** *n*-Hexane and isopropyl alcohol (99:1)

**System suitability solution:** 250 mg of USP Vitamin D Assay System Suitability RS in 10 mL of *n*-hexane. Heat this solution under reflux, at 60° for 1 h, and cool.

[NOTE—This solution contains cholecalciferol, precholecalciferol, and *trans*-cholecalciferol.]

**Standard stock solution:** 50 µg/mL of USP Cholecalciferol RS in *n*-hexane. [NOTE—Prepare this solution fresh daily.]

**Standard solution A:** 5 µg/mL of USP Cholecalciferol RS in *n*-hexane from the *Standard stock solution*

**Standard solution B:** Transfer a 5-mL volume of the *Standard stock solution* to a container having a polytetrafluoroethylene-lined screw cap. Displace the air with nitrogen and heat at 60° for 1 h under a nitrogen atmosphere, and cool. Quantitatively transfer the solution to a 50-mL volumetric flask, and dilute with *n*-hexane to volume.

**Sample solution:** Weigh NLT 30 Capsules in a tared weighing bottle. With a sharp blade or by other appropriate means, carefully open the Capsules, without loss of the shell material, and transfer as much as possible of the combined Capsule contents to a suitable container. Remove any adhering substance from the emptied Capsules and shell remains by washing with several small portions of *n*-hexane. Discard the washings, and allow the empty Capsules and shell remains to dry in a current of dry air until the odor of *n*-hexane is no longer perceptible. Weigh the empty Capsules and shell remains in the original tared weighing bottle, and calculate the average net weight per Capsule by difference. Dissolve a portion of the combined Capsule contents in *n*-hexane to prepare a cholecalciferol solution with a nominal concentration of 5 µg/mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 4.6-mm × 15-cm; 3-µm packing L8

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for precholecalciferol, *trans*-cholecalciferol, and cholecalciferol are 0.5, 0.6, and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1 between the precholecalciferol and *trans*-cholecalciferol peaks

**Relative standard deviation:** NMT 2.0% for the cholecalciferol peak

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

#### Cholecalciferol response factor

Calculate the cholecalciferol response factor,  $F_C$ :

$$F_C = C_s/r_s$$

$C_s$  = concentration of USP Cholecalciferol RS in *Standard solution A* (µg/mL)

$r_s$  = peak area of cholecalciferol from *Standard solution A*

#### Precholecalciferol response factor

Calculate the concentration,  $C'_s$ , in µg/mL, of cholecalciferol in *Standard solution B*:

$$C'_s = F_C \times r'_s$$

$F_C$  = cholecalciferol response factor

$r'_s$  = peak area of cholecalciferol from *Standard solution B*

Calculate the concentration,  $C'_{pre}$ , in µg/mL, of precholecalciferol in *Standard solution B*:

$$C'_{pre} = C_s - C'_s$$



- $C_s$  = concentration of USP Cholecalciferol RS in *Standard solution A* ( $\mu\text{g/mL}$ )  
 $C'_s$  = concentration of cholecalciferol in *Standard solution B* ( $\mu\text{g/mL}$ )

Calculate the response factor,  $F_{pre}$ , for precholecalciferol:

$$F_{pre} = C'_{pre}/r_p$$

- $C'_{pre}$  = concentration of precholecalciferol ( $\mu\text{g/mL}$ )  
 $r_p$  = peak response of precholecalciferol from *Standard solution B*

#### Content of vitamin D

Calculate the percentage of the labeled amount of vitamin D as cholecalciferol ( $\text{C}_{27}\text{H}_{44}\text{O}$ ) in the portion of Capsules taken:

$$\text{Result} = \{[(F_c \times r_c) + (F_{pre} \times r_{pre})]/C_U\} \times 100$$

- $F_c$  = response factor for cholecalciferol  
 $r_c$  = peak area of cholecalciferol from the *Sample solution*  
 $F_{pre}$  = response factor for precholecalciferol  
 $r_{pre}$  = peak area of precholecalciferol from the *Sample solution*  
 $C_U$  = nominal concentration of cholecalciferol in the *Sample solution* ( $\mu\text{g/mL}$ )

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISINTEGRATION (701)

**Buffer solution:** 0.05 M acetate buffer, prepared by mixing 2.99 g of sodium acetate and 1.66 mL of glacial acetic acid with water to obtain a 1000-mL solution having a pH of  $4.5 \pm 0.05$

**Immersion fluid:** *Buffer solution*

**Time:** 45 min

**Acceptance criteria:** Meet the requirements

##### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label the Capsules to indicate the content of cholecalciferol, in mg. The activity may be expressed also in terms of USP Units, on the basis that 40 USP Vitamin D Units = 1  $\mu\text{g}$ .
- **USP REFERENCE STANDARDS (11)**  
 USP Cholecalciferol RS  
 USP Vitamin D Assay System Suitability RS

## Cholecalciferol Solution

#### DEFINITION

Cholecalciferol Solution is a solution of Cholecalciferol in an edible vegetable oil, in Polysorbate 80, or in Propylene Glycol. It contains NLT 90.0% and NMT 120.0% of the labeled amount of vitamin D as cholecalciferol ( $\text{C}_{27}\text{H}_{44}\text{O}$ ).

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Hexane and pentanol (997:3)

**Standard stock solution:** Dissolve USP Cholecalciferol RS in toluene, and dilute with *Mobile phase* to 50  $\mu\text{g/mL}$ . [NOTE—Prepare this solution fresh daily.]

**Standard solution A:** 5  $\mu\text{g/mL}$  from *Standard stock solution* in *Mobile phase*. [NOTE—Store at a temperature not above  $0^\circ$ .]

**Standard solution B:** Transfer 5.0 mL of *Standard stock solution* to a round-bottom flask fitted with a reflux condenser. Displace the air with nitrogen, and reflux for 1

h in a water bath under a nitrogen atmosphere to obtain a solution containing cholecalciferol and precholecalciferol. Cool, transfer the solution with the aid of several portions of *Mobile phase* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample solution:** Equivalent to 5  $\mu\text{g/mL}$  of cholecalciferol in *Mobile phase* from an accurately measured volume of Cholecalciferol Solution

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L3

**Flow rate:** 2 mL/min

**Injection size:** 10  $\mu\text{L}$

#### System suitability

**Sample:** *Standard solution B*

[NOTE—The relative retention times for precholecalciferol and cholecalciferol are about 0.4 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.0 between the precholecalciferol peak and the cholecalciferol peak

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

#### Cholecalciferol response factor

Calculate the cholecalciferol response factor,  $F_c$ :

$$F_c = C_s/r_s$$

- $C_s$  = concentration of USP Cholecalciferol RS in *Standard solution A* ( $\mu\text{g/mL}$ )  
 $r_s$  = peak area of cholecalciferol from *Standard solution A*

#### Pre-cholecalciferol response factor

Calculate the concentration,  $C'_s$ , in  $\mu\text{g/mL}$ , of cholecalciferol in *Standard solution B*:

$$C'_s = F_c \times r'_s$$

- $F_c$  = response factor for cholecalciferol  
 $r'_s$  = peak area of cholecalciferol from *Standard solution B*

Calculate the concentration,  $C'_{pre}$ , in  $\mu\text{g/mL}$ , of precholecalciferol:

$$C'_{pre} = C_s - C'_s$$

- $C_s$  = concentration of USP Cholecalciferol RS in *Standard solution A* ( $\mu\text{g/mL}$ )  
 $C'_s$  = concentration of cholecalciferol in *Standard solution B* ( $\mu\text{g/mL}$ )

Calculate the response factor,  $F_{pre}$ , for precholecalciferol:

$$F_{pre} = C'_{pre}/r_p$$

- $C'_{pre}$  = concentration of precholecalciferol ( $\mu\text{g/mL}$ )  
 $r_p$  = peak response of precholecalciferol from *Standard solution B*

#### Content of vitamin D

Calculate the percentage of the labeled amount of vitamin D as cholecalciferol ( $\text{C}_{27}\text{H}_{44}\text{O}$ ) in the portion of the Cholecalciferol Solution taken:

$$\text{Result} = \{[(F_c \times r_c) + (F_{pre} \times r_{pre})]/C_U\} \times 100$$

- $F_c$  = response factor for cholecalciferol  
 $r_c$  = peak area of cholecalciferol from the *Sample solution*  
 $F_{pre}$  = response factor for precholecalciferol  
 $r_{pre}$  = peak area of precholecalciferol from the *Sample solution*



$C_U$  = nominal concentration of cholecalciferol in the  
Sample solution ( $\mu\text{g/mL}$ )

Acceptance criteria: 90.0%–120.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **LABELING:** Label it to indicate the concentration, in  $\text{mg/mL}$ , of cholecalciferol. Label it also to state that it is to be used for manufacturing only.
- **USP REFERENCE STANDARDS (11)**  
USP Cholecalciferol RS

### Cholestyramine Resin

Cholestyramine.

Cholestyramine [11041-12-6].

» Cholestyramine Resin is a strongly basic anion-exchange resin in the chloride form, consisting of styrene-divinylbenzene copolymer with quaternary ammonium functional groups. Each g exchanges not less than 1.8 g and not more than 2.2 g of sodium glycocholate, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards (11)**—

USP Cholestyramine Resin RS

**Identification**—Infrared Absorption (197K).

**pH (791):** between 4.0 and 6.0, in a slurry (1 in 100).

**Loss on drying (731)**—Dry over phosphorus pentoxide at a pressure not exceeding 50 mm of mercury at 70° for 16 hours: it loses not more than 12.0% of its weight.

**Residue on ignition (281):** not more than 0.1%.

#### Delete the following:

• **Heavy metals, Method II (231):** 0.002%. • (Official, 1-Jan-2018)

#### Dialyzable quaternary amines—

**pH 9.2 Buffer**—Transfer 3.80 g of sodium borate decahydrate to a 1000-mL volumetric flask, dissolve in and dilute with water to volume, and mix.

**Bromothymol blue solution**—Transfer 150 mg of bromothymol blue and 405 mg of sodium carbonate to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Standard solution**—Take 1 mL of 60% benzyltrimethylammonium chloride solution, accurately pipetted, and dilute quantitatively, and stepwise, with water to obtain a stock solution having a concentration of  $0.2 \pm 0.01 \text{ mg per mL}$  [NOTE—Prepare this solution fresh]. Cut a 20- to 25-cm piece of cellulose dialysis tubing\* having a molecular weight cut-off that falls within the 6,000 to 14,000 range and a dry flat width of 5 to 9 cm, and place it in water to hydrate until pliable, appropriately sealing one end. Pipet 5 mL of the stock solution into the tubing, add 5 mL of water, appropriately seal the open end, place the tube in a suitable vessel containing 100 mL of water so that it is completely immersed in the water, and stir the fluid for 16 hours to effect dialysis.

**Test solution**—Cut a 20- to 25-cm piece of cellulose dialysis tubing\* having a molecular weight cut-off that falls within the 6,000 to 14,000 range and a dry flat width of 5 to 9 cm, and place it in water to hydrate until pliable, ap-

propriately sealing one end. Weigh  $2 \pm 0.01 \text{ g}$  of Cholestyramine Resin, and carefully transfer the specimen into the tubing, taking care to ensure that none adheres to the upper walls of the tubing. Add 10 mL of water to the contents of the tube, appropriately seal the open end, and place the tube in a suitable vessel containing 100 mL of water so that it is completely immersed in the water. Stir the fluid for 16 hours to effect dialysis.

**Procedure**—Pipet the following into each of three separators: Separator 1—5 mL of *Standard solution*, 5 mL of pH 9.2 Buffer, 1 mL of *Bromothymol blue solution*, and 10 mL of chloroform; Separator 2—5 mL of *Test solution*, 5 mL of pH 9.2 Buffer, 1 mL of *Bromothymol blue solution*, and 10 mL of chloroform; Separator 3—5 mL of water, 5 mL of pH 9.2 Buffer, 1 mL of *Bromothymol blue solution*, and 10 mL of chloroform. Shake each separator vigorously for 1 minute, allow the phases to separate until the chloroform phase is clear, and collect the chloroform extracts in separate 25-mL volumetric flasks. Repeat the extraction process with a second 10-mL portion of chloroform, and combine with the previous extracts. Dilute each solution with chloroform to volume, if necessary, and mix. Concomitantly determine the absorbances of the *Test solution* and the *Standard solution* at the wavelength of maximum absorbance at about 420 nm, with a suitable spectrophotometer, using the solution from Separator 3 as the blank: the absorbance of the *Test solution* does not exceed that of the *Standard solution* (0.05% as benzyltrimethylammonium chloride).

**Chloride content**—To about 750 mg of Cholestyramine Resin, accurately weighed, add 100 mL of water and 50 mg of potassium nitrate. Add, with stirring, 2 mL of nitric acid, and titrate with 0.1 N silver nitrate VS, determining the endpoint potentiometrically, and using a silver-glass electrode system. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of Cl. Not less than 13.0% and not more than 17.0% of Cl, calculated on the dried basis, is found.

#### Exchange capacity—

**Mobile phase**—Prepare a filtered and degassed mixture of 0.08 M monobasic potassium phosphate and acetonitrile (65:35). Adjust with phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Potassium phosphate buffer**—Transfer about 4 g of monobasic potassium phosphate and about 12 g of dibasic potassium phosphate to a 1-liter volumetric flask. Dissolve in and dilute with water to volume, and mix.

**Sodium glycocholate solution**—Transfer about 15 g of sodium glycocholate to a 500-mL volumetric flask, and dissolve in and dilute with *Potassium phosphate buffer* to volume.

**Reference solution**—Pipet 4.0 mL of *Sodium glycocholate solution* into a 100-mL volumetric flask, and dilute with water to volume.

**Standard solution**—Transfer about 100 mg of USP Cholestyramine Resin RS, accurately weighed, to a 25-mL conical flask. Pipet 15.0 mL of *Sodium glycocholate solution* into the flask, and stir by mechanical means for 2 hours. Transfer the contents to a centrifuge tube, and centrifuge for 15 minutes. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with water to volume.

**System suitability solution**—Prepare a solution in water containing, in each mL, about 0.6 mg of sodium glycocholate and about 0.3 mg of taurodeoxycholic acid.

**Test solution**—Transfer about 100 mg of anhydrous Cholestyramine Resin, accurately weighed, to a 25-mL conical flask. Pipet 15.0 mL of *Sodium glycocholate solution* into the flask, and stir by mechanical means for 2 hours. Transfer the contents to a centrifuge tube, and centrifuge for 15 minutes. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with water to volume.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector

\*A suitable tubing is Spectra/Por 1, Item # 132 665, available from Spectrum Laboratories, Inc. (www.spectrum.com), or equivalent.



and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between sodium glycocholate and taurodeoxycholic acid is not less than 1.5. Chromatograph the *Reference solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Reference solution*, the *Standard solution*, and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of sodium glycocholate absorbed on each g of the Resin taken by the formula:

$$M(2.5r_R - r_U)W_S / (2.5r_R - r_S)W_U$$

in which  $M$  is the stated value, in mg, of sodium glycocholate absorbed per g of USP Cholestyramine Resin RS;  $r_R$ ,  $r_U$ , and  $r_S$  are the peak responses obtained from the *Reference solution*, the *Test solution*, and the *Standard solution*, respectively;  $W_U$  is the weight, in mg, of Cholestyramine Resin, calculated on the dried basis, taken to prepare the *Test solution*; and  $W_S$  is the weight, in mg, of USP Cholestyramine Resin RS taken to prepare the *Standard solution*.

## Cholestyramine for Oral Suspension

» Cholestyramine for Oral Suspension is a mixture of Cholestyramine Resin with suitable excipients and coloring and flavoring agents. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of dried cholestyramine resin.

**Packaging and storage**—Preserve in tight containers.

### USP Reference standards (11)—

USP Cholestyramine Resin RS

**Identification**—Transfer a quantity of Cholestyramine for Oral Suspension, equivalent to about 500 mg of dried cholestyramine resin, to a suitable flask, add 100 mL of 0.1 N hydrochloric acid, stir to suspend the solid, and heat on a steam bath for 10 minutes. Filter, wash the residue with three 50-mL portions of water, and dry at 70° and at a pressure not exceeding 50 mm of mercury for 16 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Cholestyramine Resin RS.

**Uniformity of dosage units** (905): meets the requirements for *Weight Variation*.

### Assay—

*Mobile phase*, *Potassium phosphate buffer*, *Sodium glycocholate solution*, *Reference solution*, *Standard solution*, *System suitability solution*, and *Chromatographic system*—Proceed as directed in the test for *Exchange capacity* under *Cholestyramine Resin*.

**Test solution**—Transfer an accurately weighed portion of Cholestyramine for Oral Suspension, equivalent to about 100 mg of cholestyramine resin, to a 25-mL conical flask. Pipet 15.0 mL of *Sodium glycocholate solution* into the flask, and stir by mechanical means for 2 hours. Transfer the contents to a centrifuge tube, and centrifuge for 15 minutes. Transfer 5.0 mL of the supernatant to a 50-mL volumetric flask, and dilute with water to volume.

**Procedure**—Separately inject equal volumes (about 50  $\mu$ L) of the *Reference solution*, the *Standard solution*, and the *Test*

*solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cholestyramine resin per mg of Cholestyramine for Oral Suspension taken by the formula:

$$[M(2.5r_R - r_U)W_S] / [(2.5r_R - r_S)W_UQ]$$

in which  $M$  is the stated value, in mg, of sodium glycocholate absorbed per g of USP Cholestyramine Resin RS;  $r_R$ ,  $r_U$ , and  $r_S$  are the peak responses obtained from the *Reference solution*, the *Test solution*, and the *Standard solution*, respectively;  $W_S$  is the weight, in mg, of USP Cholestyramine Resin RS taken to prepare the *Standard solution*;  $W_U$  is the weight, in mg, of Cholestyramine for Oral Suspension taken to prepare the *Test solution*; and  $Q$  is the quantity of sodium glycocholate absorbed per g of dried cholestyramine resin, as obtained in the test for *Exchange capacity* under *Cholestyramine Resin*.

## Chromic Chloride

$\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$  266.45

Chromic chloride ( $\text{CrCl}_3$ ) hexahydrate;  
Chromium(3+) chloride hexahydrate [10060-12-5].

$\text{CrCl}_3$  158.36

Anhydrous [10025-73-7].

### DEFINITION

Chromic Chloride contains NLT 98.0% and NMT 101.0% of  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ .

### IDENTIFICATION

#### • A.

**Analysis:** To 5 mL of a solution (1 in 250) in a test tube, add 1 mL of 5 N sodium hydroxide and 10 drops of 30% hydrogen peroxide, and heat gently for about 2 min.

**Acceptance criteria:** A yellow color develops.

#### • B.

**Analysis:** To 5 mL of a solution (1 in 250) in a test tube, add 5 drops of silver nitrate TS.

**Acceptance criteria:** A white, curdy precipitate that is insoluble in nitric acid is formed.

### ASSAY

#### • PROCEDURE

**Sample solution:** Dissolve 0.4 g of Chromic Chloride in 100 mL of water contained in a glass-stoppered, 500-mL conical flask. Add 5 mL of 5 N sodium hydroxide, and mix. Pipet slowly 4 mL of 30% hydrogen peroxide into the flask, and boil the solution for 5 min. Cool the solution slightly, and add 5 mL of nickel sulfate solution (1 in 20). Boil the solution until no more oxygen is evolved. Cool, and add 2 N sulfuric acid dropwise until the color of the solution changes from yellow to orange. Add to the flask a freshly prepared solution of 4 g of potassium iodide and 2 g of sodium bicarbonate in 100 mL of water, then add 6 mL of hydrochloric acid. Immediately insert the stopper in the flask, and allow to stand in the dark for 10 min. Rinse the stopper and the sides of the flask with a few mL of water.

**Analysis:** Titrate the liberated iodine with 0.1 N sodium thiosulfate VS to an orange color. Add 3 mL of starch TS, and continue the titration to a blue-green endpoint. Each mL of 0.1 N sodium thiosulfate is equivalent to 8.882 mg of chromium chloride hexahydrate ( $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ).



Acceptance criteria: 98.0%–101.0%

### IMPURITIES

#### • CHLORIDE AND SULFATE, Sulfate (221)

**Sample solution:** Dissolve 2.0 g of Chromic Chloride in 10 mL of water. Add 1 mL of 3 N hydrochloric acid, and filter if necessary to obtain a clear solution. Wash the filter with two 5-mL portions of water, and dilute with water to 40 mL.

**Control solution:** Prepare as directed in the *Sample solution*, but use 1.0 g of the substance under test. After the filtration step, add 0.10 mL of 0.020 N sulfuric acid.

**Analysis:** To each solution add 3 mL of barium chloride TS, mix, and allow to stand overnight. Decant most of the supernatants, without disturbing the precipitates, but leaving twice the volume of liquid in the *Control solution* as in the *Sample solution*. Dilute each solution with water to 25 mL, and sonicate for 1 min.

**Acceptance criteria:** Any turbidity in the *Sample solution* does not exceed that in the *Control solution* (0.01%).

#### • IRON (241)

**Test preparation:** Dissolve 1.0 g of Chromic Chloride in 100 mL of water. Transfer 10 mL of this solution to a 100-mL color comparison tube. Dilute with water to 45 mL, add 2 mL of hydrochloric acid, and mix.

**Analysis:** Proceed as directed for *Procedure*, except add 15 mL of butyl alcohol to the *Test preparation* and the *Standard Preparation* at the same time that the *Ammonium Thiocyanate Solution* is added. Shake for 30 s, and allow the layers to separate.

**Acceptance criteria:** The color in the upper butyl alcohol layer from the *Test preparation* is not darker than that from the *Standard Preparation* (NMT 0.01%).

### SPECIFIC TESTS

#### • INSOLUBLE MATTER

**Sample:** 10 g

**Analysis:** Transfer the *Sample* to a 250-mL beaker. Add 100 mL of water, cover the beaker, and heat to boiling. Digest the hot solution on a steam bath for 30 min, and filter through a tared filtering crucible of fine porosity. Rinse the beaker with hot water, passing the rinsings through the filter, and wash the filter with hot water until the last washing is colorless. Dry the filter at 105°.

**Acceptance criteria:** The weight of the residue does not exceed 1 mg (0.01%).

#### • SUBSTANCES NOT PRECIPITATED BY AMMONIUM HYDROXIDE

**Sample:** 2.0 g

**Analysis:** Dissolve the *Sample* in 80 mL of water, heat the solution to boiling, and add a slight excess of ammonium hydroxide. Continue heating to remove the excess ammonia. Cool, dilute with water to 100.0 mL, and mix. Pass through a retentive filter, and transfer 50.0 mL of the clear filtrate to an evaporating dish that previously has been ignited and tared. Add 0.5 mL of sulfuric acid to the filtrate, and evaporate on a steam bath to dryness. Heat gently to remove the excess acid, and ignite gently.

**Acceptance criteria:** The weight of the residue does not exceed 2.0 mg (0.20% as sulfate).

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in tight containers.

contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of chromium (Cr).

**Packaging and storage—**Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

**Labeling—**Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.

#### USP Reference standards (11)—

USP Endotoxin RS

**Identification—**The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 360 nm when tested as directed for *Procedure* in the *Assay*.

**Bacterial Endotoxins Test (85)—**It contains not more than 16.70 USP Endotoxin Units per µg of chromium.

**pH (791):** between 1.5 and 2.5.

**Other requirements—**It meets the requirements under *Injections and Implanted Drug Products (1)*.

#### Assay—

**Sodium chloride solution—**Dissolve 54 g of sodium chloride in water, dilute with water to 2000 mL, and mix.

**Chromium stock solution—**Transfer 2.829 g of potassium dichromate, accurately weighed, to a 1000-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. This solution contains 1000 µg of chromium per mL. Store in a polyethylene bottle.

**Standard preparations—**Pipet 10 mL of the *Chromium stock solution* into a 1000-mL volumetric flask, dilute with water to volume, and mix. Transfer 10.0 mL and 20.0 mL, respectively, of this stock solution to separate 100-mL volumetric flasks, and transfer 15.0 mL and 20.0 mL, respectively, of the stock solution to separate 50-mL volumetric flasks. Add 20 mL of *Sodium chloride solution* to each 100-mL volumetric flask, and 10 mL of *Sodium chloride solution* to each 50-mL volumetric flask, dilute the contents of each flask with water to volume, and mix. These *Standard preparations* contain, respectively, 1.0, 2.0, 3.0, and 4.0 µg of chromium per mL.

**Assay preparation—**Transfer an accurately measured volume of Injection, equivalent to about 60 µg of chromium, to a 25-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the volume of Injection taken, and add sufficient *Sodium chloride solution* to bring the total sodium chloride content of the flask to 135 mg. Dilute with water to volume, and mix.

**Procedure—**Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the chromium emission line of 357.9 nm, with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy (852)*) equipped with a chromium hollow-cathode lamp and an air-acetylene flame, using a 1:5 dilution of the *Sodium chloride solution* as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of chromium, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, in µg per mL, of chromium in the *Assay preparation*. Calculate the quantity, in µg, of chromium in each mL of the Injection taken by the formula:

$$25C/V$$

in which C is the concentration, in µg per mL, of chromium in the *Assay preparation*, and V is the volume, in mL, of Injection taken.

## Chromic Chloride Injection

» Chromic Chloride Injection is a sterile solution of Chromic Chloride in Water for Injection. It



## Sodium Chromate Cr 51 Injection

Chromic acid ( $\text{H}_2^{51}\text{CrO}_4$ ), disodium salt.  
Disodium chromate ( $\text{Na}_2^{51}\text{CrO}_4$ ) [7775-11-3].

» Sodium Chromate Cr 51 Injection is a sterile solution of radioactive chromium ( $^{51}\text{Cr}$ ) processed in the form of sodium chromate in Water for Injection. For those uses where an isotonic solution is required, Sodium Chloride may be added in appropriate amounts as provided under *Injections and Implanted Drug Products* (1). Chromium 51 is produced by the neutron bombardment of enriched chromium 50.

Sodium Chromate Cr 51 Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{51}\text{Cr}$  as sodium chromate expressed in megabecquerels (millicuries) per mL at the time indicated in the labeling. The sodium chromate content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount. The specific activity is not less than 370 megabecquerels (10 millicuries) per mg of sodium chromate at the end of the expiry period. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

**Packaging and storage**—Preserve in single-dose or in multiple-dose containers.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* (7), *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of sodium chromate expressed in  $\mu\text{g}$  per mL; the amount of  $^{51}\text{Cr}$  as sodium chromate expressed as total megabecquerels (millicuries) and as megabecquerels (millicuries) per mL at the time of calibration; a statement to indicate whether the contents are intended for diagnostic or therapeutic use; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay and the quantity of chromium, and also indicates that the radioactive half-life of  $^{51}\text{Cr}$  is 27.8 days.

**USP Reference standards** (11)—  
USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{51}\text{Cr}$  of known purity that exhibits a photopeak having an energy of 0.320 MeV.

**Bacterial Endotoxins Test** (85)—It contains not more than 175/V USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 7.5 and 8.5.

**Radiochemical purity**—Place a volume of Injection, appropriately diluted so that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* (621)), and immediately develop with a mixture of 5 parts of water, 2 parts of dilute alcohol (9.5 in 10), and 1 part of ammonium hydroxide. Air-dry the chromatogram, and determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. The radioactivity of the chromate band is not less than 90.0% of the total radioactivity. The  $R_f$  value for the chromate band falls within  $\pm 10\%$  of the value found for a

known sodium chromate specimen when determined under identical conditions.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1); not subject to *Container content*.

### Assay for sodium chromate—

**Standard stock preparation**—Dissolve 3.735 g of potassium chromate in 1000 mL of water to obtain a solution having a known concentration of 1.0 mg per mL of chromium.

**Standard preparation**—Pipet 0.25, 0.50, 0.75, 0.100, 0.125, and 0.150 mL of the *Standard stock preparation*, accurately measured, into separate 100-mL volumetric flasks. To each flask add 0.42 mL of 0.1 N sodium bicarbonate, and dilute with water to volume to obtain solutions having final concentrations of 0.25, 0.50, 0.75, 1.00, 1.25, and 1.50  $\mu\text{g}$  of chromium per mL.

**Assay preparation**—Use the Injection.

**Blank preparation**—Transfer 0.42 mL of 0.1 N sodium bicarbonate to a 100-mL volumetric flask, and dilute with water to volume.

**Procedure**—Concomitantly determine the absorbances of the *Assay preparation*, the *Standard preparations*, and the *Blank preparation* at the chromium emission line at 357.7 nm with a suitable atomic absorption spectrophotometer (see *Atomic Absorption Spectroscopy* (852)) equipped with a chromium hollow-cathode lamp and an air-acetylene (fuel-rich) flame using water to set the instrument to zero. Plot the absorbances of the *Standard preparations* and the *Blank preparation* versus concentration, in  $\mu\text{g}$  per mL, of chromium, and perform a regression analysis. A suitable standard curve will have an intercept between  $-0.002$  and  $+0.002$ , and a regression coefficient of not less than 0.99. Using the standard curve so obtained, determine the concentration, C, in  $\mu\text{g}$  per mL, of chromium in the Injection taken. Calculate the quantity of sodium chromate, in  $\mu\text{g}$  per mL, by the formula:

$$3.115C$$

in which 3.115 is the conversion factor.

### Change to read:

**Assay for radioactivity**—Using a suitable counting assembly (CN 1-May-2017), determine the radioactivity, in MBq ( $\mu\text{Ci}$ ) per mL, of Injection by use of a calibrated system as directed under *Radioactivity* (821).

## Chromium Cr 51 Edetate Injection

Glycine, N,N'-1,2-ethanedithiolbis[N-(carboxymethyl)], chromium-51 complex.  
(Ethylenedinitrilo)tetraacetic acid, chromium-51 complex [27849-89-4].

» Chromium Cr 51 Edetate Injection is a sterile solution containing radioactive chromium ( $^{51}\text{Cr}$ ) in the form of a complex of chromium (III) with edetic acid, present in excess. It is made isotonic by the addition of Sodium Chloride. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{51}\text{Cr}$  as edetate complex expressed in megabecquerels (or microcuries or millicuries) per mL at the time indicated in the labeling. Other chemical forms of radioactivity do not exceed 5.0 percent of the



total radioactivity. It may contain a suitable preservative. It contains not more than 1 mg of chromium (Cr) per mL.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, at a temperature between 2° and 8°.

**Labeling**—Label it to include the following, in addition to the information specified for *Labeling* (7), *Labels and Labeling for Injectable Products*: the time and date of calibration; the amount of  $^{51}\text{Cr}$  as edetate complex expressed as total MBq (or mCi) and as MBq (or mCi) per mL at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay and the quantity of chromium, and also indicates that the radioactive half-life of  $^{51}\text{Cr}$  is 27.8 days.

**USP Reference standards** (11)—

USP Endotoxin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of  $^{51}\text{Cr}$  of known purity that exhibits a photopeak having an energy of 0.320 MeV.

**Bacterial Endotoxins Test** (85): not more than 175/V USP Endotoxin Unit per mL of the Injection, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

**pH** (791): between 3.5 and 6.5.

**Radiochemical purity**—

**Electrolyte solution**—Dissolve 0.2 g of barbitol sodium and 10 g of sodium nitrate in water to make 1000 mL.

**Procedure**—Soak a 2.5-cm × 17.0-cm × 0.22-mm cellulose strip in 100 mL of *Electrolyte solution* for 10 to 60 minutes. Remove the strip with forceps, and blot to remove excess solution. Attach the strip to the support bridge of an electrophoresis chamber containing *Electrolyte solution*. Apply to the strip about 10  $\mu\text{L}$  of Injection as a 3-mm band at a position 10 cm from the cathode. Attach the chamber cover, and perform the electrophoresis at 30 V per cm, using a stabilized current. Remove the strip from the chamber, and blot the ends. Using a suitable scanner and counting assembly, determine the radioactivity distribution: chromium  $^{51}\text{Cr}$  edetate moves about 5 cm towards the anode; and  $^{51}\text{Cr}$  chromic ion moves about 7 cm towards the cathode. The radioactivity of the chromium  $^{51}\text{Cr}$  edetate band is not less than 95% of the total radioactivity.

**Change to read:**

**Radionuclidic purity** (see *Radioactivity* (821)) (CN 1-May-2017)—Using a suitable gamma-ray spectrometer (CN 1-May-2017), determine the radioactivity of each radionuclidic impurity observed in the gamma-ray spectrum: not more than 0.1% of any individual impurity is found; and not more than 0.3% of total impurities is found.

**Chemical purity**—Using a validated limit test and a known analytical technique, demonstrate the absence of any ingredients and reagents employed in the synthetic process.

**Limit of free chromium**—

**Standard solution**—Dissolve 0.96 g of chromium potassium sulfate dodecahydrate and 2.87 g of edetate disodium in 50 mL of water, boil for 10 minutes, cool, adjust with 0.2 M sodium hydroxide to a pH between 3.5 and 6.5, and dilute with water to 100.0 mL to obtain a solution having a known concentration of about 1 mg of chromium per mL.

**Test solution**—Use the Injection.

**Procedure**—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* at the wavelength of maximum absorbance at about 560 nm, with a suitable spectrophotometer, using water as the blank: the absorb-

ance of the *Test solution* is not more than that of the *Standard solution*.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1), except that it is not subject to the recommendation on *Container Content*.

**Change to read:**

**Assay for radioactivity** (see *Radioactivity* (821)) (CN 1-May-2017)—Using a suitable counting assembly (CN 1-May-2017), determine the radioactivity, in MBq (or  $\mu\text{Ci}$ ) per mL, of Injection by use of a calibrated system.

## Chymotrypsin

Chymotrypsin [9004-07-3].

### DEFINITION

Chymotrypsin is a proteolytic enzyme crystallized from an extract of the pancreas gland of the ox, *Bos taurus* Linné (Fam. Bovidae). It contains NLT 1000 USP Chymotrypsin Units/mg, calculated on the dried basis, and NLT 90.0% and NMT 110.0% of the labeled potency, as determined by the Assay.

### ASSAY

#### PROCEDURE

**Monobasic potassium phosphate solution:** 9.08 mg/mL of monobasic potassium phosphate in water

**Dibasic sodium phosphate solution:** 9.46 mg/mL of anhydrous dibasic sodium phosphate in water

**Phosphate buffer:** Mix 38.9 mL of *Monobasic potassium phosphate solution* and 61.1 mL of *Dibasic sodium phosphate solution*. If necessary, adjust to a pH of 7.0 by the dropwise addition of *Dibasic sodium phosphate solution*.

**Substrate solution:** Dissolve 23.7 mg of N-acetyl-L-tyrosine ethyl ester, suitable for use in assaying Chymotrypsin, in 50 mL of *Phosphate buffer*, with warming. When the solution is cool, dilute with additional *Phosphate buffer* to 100 mL. [NOTE—*Substrate solution* may be stored in the frozen state and used after thawing, but it is important to freeze it immediately after preparation.]

**Sample solution:** Dissolve a quantity of Chymotrypsin in 0.0012 N hydrochloric acid to yield a solution containing 12–16 USP Chymotrypsin Units/mL. The dilution is correct if, during the conduct of the Assay, there is a change in absorbance of between 0.008 and 0.012 in each 30-s interval.

#### Analysis

[NOTE—Determine the suitability of the substrate and check the adjustment of the spectrophotometer by performing the *Analysis* using USP Chymotrypsin RS in place of the *Sample solution*.]

Conduct the Assay in a suitable spectrophotometer equipped to maintain a temperature of  $25 \pm 0.1^\circ$  in the cell compartment. Determine the temperature in the reaction cell before and after the absorbance measurement to ensure that the temperature does not change by more than  $0.5^\circ$ . Pipet 0.2 mL of 0.0012 N hydrochloric acid and 3.0 mL of *Substrate solution* into a 1-cm cell. Place the cell in the spectrophotometer, and adjust the instrument so that the absorbance will read 0.200 at 237 nm. Pipet 0.2 mL of *Sample solution* into another 1-cm cell, add 3 mL of *Substrate solution*, and place the cell in the spectrophotometer. [NOTE—Carefully follow this order of addition, and begin timing the reaction from the addition of the *Substrate solution*.] Read the absorbance at 30-s intervals for NLT 5 min. Repeat the procedure on the same dilution at least once. Absolute absorbance values are less important than a constant rate of absorbance change. If the



rate of change fails to remain constant for NLT 3 min, repeat the test and, if necessary, use a lower concentration. The duplicate determination of the *Sample solution* matches the first determination, of the same dilution, in rate of absorbance change.

Determine the average absorbance change per min, using only the values within the 3-min portion of the curve where the rate of absorbance change is constant. Plot a curve of absorbance against time. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075/min under the conditions specified in the *Assay*.

Calculate the number of USP Chymotrypsin Units/mg in the portion of Chymotrypsin taken:

$$\text{Result} = (A_2 - A_1)/(T \times W \times F)$$

- $A_2$  = absorbance straight-line initial reading  
 $A_1$  = absorbance straight-line final reading  
 $T$  = time elapsed between the initial and final readings (min)  
 $W$  = weight of Chymotrypsin in the volume of solution used in determining the absorbance (mg)  
 $F$  = Chymotrypsin activity conversion factor, 0.0075/min

**Acceptance criteria:** NLT 1000 USP Chymotrypsin Units/mg on the dried basis; 90.0%–110.0% of the labeled potency

#### IMPURITIES

• **RESIDUE ON IGNITION** (281): NMT 2.5%

#### • LIMIT OF TRYPSIN

**Tris buffer:** Dissolve 294 mg of calcium chloride in 40 mL of 0.20 M tris(hydroxymethyl)aminomethane. Adjust with 1 N hydrochloric acid to a pH of 8.1, and dilute with water to 100 mL.

**Substrate solution:** Transfer 98.5 mg of *p*-toluenesulfonyl-L-arginine methyl ester hydrochloride, suitable for use in assaying trypsin, to a 25-mL volumetric flask. Add 5 mL of *Tris buffer*, and swirl until the substrate dissolves. Add 0.25 mL of methyl red–methylene blue TS, and dilute with water to volume.

**Sample solution:** 10 mg/mL of Chymotrypsin in water

**Analysis**  
 [NOTE—Determine the suitability of the substrate by performing the *Analysis* using the appropriate amount of USP Trypsin Crystallized RS in place of the *Sample solution*.]

By means of a micropipet, transfer 50  $\mu$ L of *Sample solution* to a depression on a white spot plate. Add 0.2 mL of *Substrate solution*.

**Acceptance criteria:** No purple color develops within 3 min (NMT 1% of trypsin).

#### SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): It meets the requirements of the tests for absence of *Pseudomonas aeruginosa*, *Salmonella* species, and *Staphylococcus aureus*.

#### • LOSS ON DRYING (731)

**Analysis:** Dry in a vacuum oven at 60° for 4 h.

**Acceptance criteria:** NMT 5.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.

#### • USP REFERENCE STANDARDS (11)

USP Chymotrypsin RS

USP Trypsin Crystallized RS

## Chymotrypsin for Ophthalmic Solution

#### DEFINITION

Chymotrypsin for Ophthalmic Solution is sterile Chymotrypsin. When constituted as directed in the labeling, it yields a solution containing NLT 80.0% and NMT 120.0% of the labeled potency.

#### IDENTIFICATION

##### • PROCEDURE

**Monobasic potassium phosphate solution:** 9.08 mg/mL of monobasic potassium phosphate in water

**Dibasic sodium phosphate solution:** 9.46 mg/mL of anhydrous dibasic sodium phosphate in water

**Phosphate buffer:** Mix 38.9 mL of *Monobasic potassium phosphate solution* and 61.1 mL of *Dibasic sodium phosphate solution*. If necessary, adjust to a pH of 7.0 by the dropwise addition of *Dibasic sodium phosphate solution*.

**Substrate solution:** Transfer 237.0 mg of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in assaying chymotrypsin, to a 100-mL volumetric flask, add 2 mL of alcohol, and swirl until solution is effected. Add 20 mL of *Phosphate buffer*, 1 mL of methyl red–methylene blue TS, and dilute with water to volume. If necessary, adjust to a pH of 7.0 by the dropwise addition of *Monobasic potassium phosphate solution*.

**Sample solution:** Dissolve the contents of 1 vial of Chymotrypsin for Ophthalmic Solution in 1 mL of saline TS.

**Analysis:** Transfer 0.2 mL of *Sample solution* to a suitable dish, and add 0.2 mL of *Substrate solution*.

**Acceptance criteria:** A purple color is produced within 3 min.

[NOTE—This is distinct from trypsin, which produces no purple color within 3 min.]

#### ASSAY

##### • PROCEDURE

**Monobasic potassium phosphate solution:** 9.08 mg/mL of monobasic potassium phosphate in water

**Dibasic sodium phosphate solution:** 9.46 mg/mL of anhydrous dibasic sodium phosphate in water

**Phosphate buffer:** *Monobasic potassium phosphate solution* and *Dibasic sodium phosphate solution* (38.9: 61.1). If necessary, adjust to a pH of 7.0 by the dropwise addition of *Dibasic sodium phosphate solution*.

**Substrate solution:** Dissolve 23.7 mg of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in assaying chymotrypsin, in 50 mL of *Phosphate buffer*, with warming. When the solution is cool, dilute with additional *Phosphate buffer* to 100 mL. [NOTE—*Substrate solution* may be stored in the frozen state and used after thawing, but it is important to freeze it immediately after preparation.]

**Sample stock solution:** Dissolve the contents of 1 vial of Chymotrypsin for Ophthalmic Solution in 5.0 mL of 0.0012 N hydrochloric acid.

**Sample solution:** Dilute a volume ( $V_2$ , in mL) of the *Sample stock solution*, equivalent to 300 USP Chymotrypsin Units, with 0.0012 N hydrochloric acid to 25.0 mL. The dilution is correct if, during the conduct of the *Assay*, there is a change in absorbance of between 0.008 and 0.012 in each 30-s interval.

##### Analysis

[NOTE—Determine the suitability of the substrate and check the adjustment of the spectrophotometer by performing the *Analysis* using USP Chymotrypsin RS in place of the *Sample solution*.]



Conduct the Assay in a suitable spectrophotometer equipped to maintain a temperature of  $25 \pm 0.1^\circ$  in the cell compartment. Determine the temperature in the reaction cell before and after the absorbance measurement to ensure that the temperature does not change by more than  $0.5^\circ$ . Pipet 0.2 mL of 0.0012 N hydrochloric acid and 3.0 mL of *Substrate solution* into a 1-cm cell. Place the cell in the spectrophotometer, and adjust the instrument so that the absorbance will read 0.200 at 237 nm. Pipet 0.2 mL of *Sample solution* into another 1-cm cell, add 3 mL of *Substrate solution*, and place the cell in the spectrophotometer. [NOTE—Carefully follow this order of addition, and begin timing the reaction from the addition of the *Substrate solution*.] Read the absorbance at 30-s intervals for NLT 5 min. Repeat the procedure on the same dilution at least once. Absolute absorbance values are less important than a constant rate of absorbance change. If the rate of change fails to remain constant for NLT 3 min, repeat the test and, if necessary, use a lower concentration. The duplicate determination at the same dilution matches the first determination in rate of absorbance change.

Determine the average absorbance change per min, using only the values within the 3-min portion of the curve where the rate of absorbance change is constant. Plot a curve of absorbance against time. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075/min under the conditions specified in the Assay.

Calculate the percentage of label potency of USP Chymotrypsin Units in a vial:

$$\text{Result} = [F_1 \times (V_1/V_2) \times (A_2 - A_1)] / (T \times F_2 \times F_3)$$

- $F_1$  = total USP Chymotrypsin Units in the *Sample solution*, 300  
 $V_1$  = volume of the *Sample stock solution*, 5 mL  
 $V_2$  = volume as defined in the *Sample solution* (mL)  
 $A_2$  = absorbance straight-line initial reading  
 $A_1$  = absorbance straight-line final reading  
 $T$  = time elapsed between the initial and final readings (min)  
 $F_2$  = number of USP Chymotrypsin Units in the solution on which the absorbance was determined, 2.4  
 $F_3$  = Chymotrypsin activity conversion factor, 0.0075/min

**Acceptance criteria:** 80.0%–120.0% of the labeled potency.

#### PERFORMANCE TESTS

##### • UNIFORMITY OF DOSAGE UNITS (905)

**Analysis:** Assay 10 individual units as directed in the Assay, and calculate the average of the 10 results.

**Acceptance criteria:** Meets the requirements of the chapter and the average is 80.0%–120.0% of the labeled potency. The contents of NMT 2 vials deviate by more than 10% from the average content. The contents of none of the vials deviate by more than 15% from the average.

#### IMPURITIES

##### • LIMIT OF TRYPSIN

**Tris buffer:** Dissolve 294 mg of calcium chloride in 40 mL of 0.20 M tris(hydroxymethyl)aminomethane. Adjust with 1 N hydrochloric acid to a pH of 8.1, and dilute with water to 100 mL.

**Substrate solution:** Transfer 98.5 mg of *p*-toluenesulfonyl-L-arginine methyl ester hydrochloride, suitable for use in assaying trypsin, to a 25-mL volumetric flask. Add 5 mL of *Tris buffer*, and swirl until the substrate dissolves. Add 0.25 mL of methyl red–methylene blue TS, and dilute with water to volume.

**Sample:** 10 mg/mL of Chymotrypsin for Ophthalmic Solution

#### Analysis

[NOTE—Determine the suitability of the substrate by performing the *Analysis* using the appropriate amount of USP Trypsin Crystallized RS in place of the *Sample*.] By means of a micropipet, transfer 50  $\mu$ L of the *Sample* to a depression on a white spot plate. Add 0.2 mL of the *Substrate solution*.

**Acceptance criteria:** No purple color develops within 3 min (NMT 1% of trypsin).

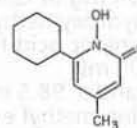
#### SPECIFIC TESTS

- **pH (791):** 4.3–8.7, in the solution constituted as directed in the labeling
- **STERILITY TESTS (71):** Meets the requirements
- **COMPLETENESS OF SOLUTION (641):** It dissolves in the solvent and in the concentration recommended in the labeling to yield a clear solution.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, and avoid exposure to excessive heat.
- **USP REFERENCE STANDARDS (11)**  
 USP Chymotrypsin RS  
 USP Trypsin Crystallized RS

## Ciclopirox



$C_{12}H_{17}NO_2$  207.27  
 2(1*H*)-Pyridinone, 6-cyclohexyl-1-hydroxy-4-methyl-  
 6-Cyclohexyl-1-hydroxy-4-methyl-2(1*H*)-pyridone  
 [29342-05-0].

» Ciclopirox contains not less than 98.0 percent and not more than 101.0 percent of  $C_{12}H_{17}NO_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers, protected from light. Store at a temperature between  $15^\circ$  and  $30^\circ$ .

#### USP Reference standards (11)—

- USP Ciclopirox RS
- USP Ciclopirox Related Compound A RS  
 3-Cyclohexyl-4,5-dihydro-5-methyl-5-isoxazolyl acetic acid.
- USP Ciclopirox Related Compound B RS  
 6-Cyclohexyl-4-methyl-2-pyrone.

**Identification, Infrared Absorption (197K).**

**Loss on drying (731)**—Dry it in vacuum to constant weight: it loses not more than 1.5% of its weight.

**Residue on ignition (281):** not more than 0.1%.

#### Delete the following:

• **Heavy metals, Method II (231):** not more than 0.001%.

• (Official 1-Jan-2018)

**Related compounds**—[NOTE—Carry out the operations avoiding exposure to actinic light. All materials in direct connection with Ciclopirox, like column materials, reagents, solvents, and others should contain only very low amounts of extractable metal cations.]



**Mobile phase**—Prepare a filtered and degassed mixture of an edetate disodium solution (0.96 in 1000), acetonitrile, and glacial acetic acid (770:230:0.1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Rinsing solution**—Prepare a mixture of water, acetonitrile, glacial acetic acid, and acetylacetone (500 : 500 : 1:1).

**Standard stock solution**—Dissolve USP Ciclopirox Related Compound A RS and USP Ciclopirox Related Compound B RS, accurately weighed, in an appropriate volume of acetonitrile and *Mobile phase* solution (approximate ratio, 1:7). Further dilute with *Mobile phase* to obtain a solution having a known final concentration of about 1.5 mg each per mL.

**Standard solution A**—Dilute 1.0 mL of *Standard stock solution* to 200.0 mL with a mixture of *Mobile phase* and acetonitrile (9:1).

**Standard solution B**—Dilute 2.0 mL of *Standard solution A* to 10.0 mL with a mixture of *Mobile phase* and acetonitrile (9:1).

**Test solution**—Dissolve 30 mg of Ciclopirox, accurately weighed, in a mixture of 2 mL of acetonitrile and 15 mL of *Mobile phase*. If necessary, use an ultrasonic bath. Dilute with *Mobile phase* to 20.0 mL.

**Resolution solution**—Mix 5 mL of *Standard stock solution* with 5 mL of the *Test solution*.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a detector capable of recording at both 220 nm and 298 nm and a 4.0-mm × 8-cm column that contains packing L10. [NOTE—Ciclopirox related compound A has an intense absorbance at 220 nm, and 6-cyclohexyl-4-methyl-2(1*H*)-pyridone, ciclopirox related compound B, and ciclopirox have intense absorbances at 298 nm.] The flow rate is about 0.7 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure* at 298 nm: the resolution, *R*<sub>s</sub>, between the ciclopirox related compound B peak and ciclopirox peak is not less than 2.0. Chromatograph the *Standard solution B*, and record the peak responses as directed for *Procedure* at 298 nm: the chromatogram obtained shows at 298 nm a peak corresponding to ciclopirox related compound B with a signal-to-noise ratio of not less than 3. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure* at 298 nm: the tailing factor for the ciclopirox peak is not less than 2.0.

**Procedure**—Separately inject equal volumes (about 10 µL) of *Standard solution A*, *Standard solution B*, and the *Test solution* into the chromatograph, and record the chromatograms. [NOTE—In order to ensure desorption of disruptive metal ions, every new column must be rinsed with the *Rinsing solution* over a period of not less than 15 hours and then with the *Mobile phase* for not less than 5 hours with a flow rate of 0.2 mL per minute. The chromatographic run time is not less than 2.5 times the retention time of the ciclopirox peak.] The relative retention times are about 0.5 for ciclopirox related compound A, 0.9 for 6-cyclohexyl-4-methyl-2(1*H*)-pyridone, 1.0 for ciclopirox, and 1.3 for ciclopirox related compound B. The peak response at 220 nm of the ciclopirox related compound A peak in the chromatogram obtained from the *Test solution* is not more than the peak response at 220 nm of the corresponding peak in the chromatogram obtained from *Standard solution A* (0.5%). The sum of responses at 298 nm of the peaks in the chromatogram obtained from the *Test solution* is not more than the peak response at 298 nm of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution A* (0.5%). At 298 nm disregard any peak due to the solvent and any peak with a response less than the response of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution B* at 298 nm (0.1%).

**Assay**—Dissolve 150 mg of Ciclopirox, accurately weighed, in 20 mL of methanol. Add 20 mL of water, mix, and titrate

with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Carry out a blank test. Determine the factor of the 0.1 N sodium hydroxide VS using 100 mg of benzoic acid, accurately weighed, and titrate under the conditions prescribed above. Each mL of 0.1 N sodium hydroxide is equivalent to 20.73 mg of C<sub>12</sub>H<sub>17</sub>NO<sub>2</sub>.

## Ciclopirox Topical Solution

### DEFINITION

Ciclopirox Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of ciclopirox (C<sub>12</sub>H<sub>17</sub>NO<sub>2</sub>).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

[NOTE—Protect the *Standard solution* and *Sample solution* from light.]

**Buffer:** Transfer 5.25 g of citric acid and 25 mL of 0.1 M edetate disodium to a 1-L volumetric flask, and dilute with water to volume. Adjust with 8.5% diluted sodium hydroxide solution to a pH of 6.5.

**Mobile phase:** Acetonitrile and *Buffer* (35:65)

**Standard solution:** 0.2 mg/mL of USP Ciclopirox RS and 1 µg/mL each of USP Ciclopirox Related Compound B RS and USP Ciclopirox Related Compound C RS in methanol

**Sample solution:** Equivalent to 0.2 mg/mL of ciclopirox in methanol from Topical Solution. Pass through a filter of 0.45-µm pore size, and use the filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 303 nm

**Column:** 4-mm × 12.5-cm; 5-µm packing L1

**Column temperature:** 30 ± 5°

**Flow rate:** 0.9 mL/min

**Run time:** 5 times the retention time of the major peak

**Injection size:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—For information only, see *Table 1* for relative retention times of impurities.]

#### Suitability requirements

**Resolution:** NLT 3.0 between ciclopirox and ciclopirox related compound B; and NLT 3.0 between ciclopirox related compound C and ciclopirox

**Tailing factor:** NMT 2.0 for the ciclopirox peak

**Relative standard deviation:** NMT 2.0% for the ciclopirox peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ciclopirox (C<sub>12</sub>H<sub>17</sub>NO<sub>2</sub>) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r*<sub>U</sub> = peak response from the *Sample solution*

*r*<sub>S</sub> = peak response from the *Standard solution*

*C*<sub>S</sub> = concentration of USP Ciclopirox RS in the *Standard solution* (mg/mL)

*C*<sub>U</sub> = nominal concentration of ciclopirox in the *Sample solution* (mg/mL)



Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirement

## IMPURITIES

### • ORGANIC IMPURITIES

Buffer, Mobile phase, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

#### Analysis

Sample: Sample solution

Calculate the percentage of each impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_u/r_T) \times (1/F) \times 100$$

$r_u$  = peak response of each individual impurity from the Sample solution

$r_T$  = sum of responses of all the peaks in the Sample solution

$F$  = relative response factor (see Table 1)

Acceptance criteria: See Table 1.

Table 1

Compound	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Ciclopirox related compound C	0.54	1.3	0.5
Ciclopirox	1.0	—	—
Ciclopirox related compound B <sup>a</sup>	1.87	—	—
Any unspecified individual impurity	—	1.0	0.2
Total impurities	—	—	1.2

<sup>a</sup> Process impurity already monitored in the drug substance.

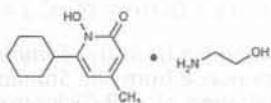
## SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed  $10^2$  cfu/g, and the total combined molds and yeasts count does not exceed  $10^1$  cfu/g.

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
  - USP Ciclopirox RS
  - USP Ciclopirox Related Compound B RS
  - 6-Cyclohexyl-4-methyl-2-pyrone.
  - $C_{12}H_{16}O_2$  192.25
  - USP Ciclopirox Related Compound C RS
  - 6-Cyclohexyl-4-methylpyridin-2(1H)-one.
  - $C_{12}H_{17}NO$  191.27

## Ciclopirox Olamine



$C_{12}H_{17}NO_2 \cdot C_2H_7NO$  268.35  
2(1H)-Pyridinone, 6-cyclohexyl-1-hydroxy-4-methyl-, compound with 2-aminoethanol (1:1).

6-Cyclohexyl-1-hydroxy-4-methyl-2(1H)-pyridone compound with 2-aminoethanol (1:1) [41621-49-2].

» Ciclopirox Olamine contains not less than 97.5 percent and not more than 101.5 percent of ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ).

**Packaging and storage**—Preserve in tight containers, protected from light. Store between 5° and 25°.

### USP Reference standards (11)—

USP Ciclopirox Olamine RS

USP Ciclopirox Related Compound A RS

3-Cyclohexyl-4,5-dihydro-5-methyl-5-isoxazolyl acetic acid.

USP Ciclopirox Related Compound B RS

6-Cyclohexyl-4-methyl-2-pyrone.

**Identification, Infrared Absorption (197K).**

**pH (791):** between 8.0 and 9.0, in a mixture with water (1:100).

**Residue on ignition (281):** not more than 0.1%.

### Delete the following:

- **Heavy metals, Method II (231):** not more than 0.001%.

• (Official 1-Jan-2018)

**Monoethanolamine content**—Dissolve about 300 mg, accurately weighed, in 25 mL of glacial acetic acid. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 6.108 mg of  $C_2H_7NO$ . The content of monoethanolamine ( $C_2H_7NO$ ) is not less than 223 mg and not more than 230 mg per g of ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ) found in the Assay.

**Related compounds**—[NOTE—Carry out the operations avoiding exposure to actinic light. All materials that are in direct contact with Ciclopirox Olamine (e.g., column materials, reagents, solvents, etc.) should contain only very low amounts of extractable metal cations.]

**Mobile phase**—Prepare a filtered and degassed mixture of an edetate disodium solution (0.96 in 1000), acetonitrile, and glacial acetic acid (770:230:0.1). Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Rinsing solution**—Prepare a mixture of water, acetonitrile, glacial acetic acid, and acetylacetone (500:500:1:1).

**Standard stock solution**—Dissolve USP Ciclopirox Related Compound A RS and USP Ciclopirox Related Compound B RS, accurately weighed, in an appropriate volume of acetonitrile and Mobile phase solution (approximate ratio, 1:7). Further dilute with Mobile phase to obtain a solution having a known final concentration of about 1.5 mg of each per mL.

**Standard solution A**—Dilute 1.0 mL of Standard stock solution to 200.0 mL with a mixture of Mobile phase and acetonitrile (9:1).

**Standard solution B**—Dilute 2.0 mL of Standard solution A to 10.0 mL with a mixture of Mobile phase and acetonitrile (9:1).

**Test solution**—Dissolve 40 mg of Ciclopirox Olamine, accurately weighed, in a mixture of 2 mL of acetonitrile, 20  $\mu$ L of glacial acetic acid, and 15 mL of Mobile phase. If necessary, use an ultrasonic bath to dissolve. Dilute with Mobile phase to 20.0 mL, and mix.

**Resolution solution**—Mix 5 mL of Standard stock solution with 5 mL of the Test solution.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a detector capable of recording at both 220 nm and 298 nm and a 4.0-mm  $\times$  8-cm column that contains packing L10. [NOTE—Ciclopirox



related compound A has an intense absorbance at 220 nm, and 6-cyclohexyl-4-methyl-2(1*H*)-pyridone, ciclopirox related compound B, and ciclopirox have intense absorbances at 298 nm.] The flow rate is about 0.7 mL per minute. Chromatograph the *Resolution solution* at 298 nm, and record the peak responses as directed for *Procedure*: the resolution,  $R_f$  between the ciclopirox related compound B peak and the ciclopirox peak is not less than 2.0. Chromatograph *Standard solution B* at 298 nm, and record the peak responses as directed for *Procedure*: the chromatogram obtained shows at 298 nm a peak corresponding to ciclopirox related compound B with a signal-to-noise ratio of not less than 3. Chromatograph the *Test solution* at 298 nm, and record the peak responses as directed for *Procedure*: the tailing factor for the ciclopirox peak is less than 2.0.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of *Standard solution A*, *Standard solution B*, and the *Test solution* into the chromatograph, and record the chromatograms. [NOTE—In order to ensure desorption of disruptive metal ions, every new column must be rinsed with the *Rinsing solution* over a period of not less than 15 hours and then with *Mobile phase* for not less than 5 hours with a flow rate of 0.2 mL per minute. The chromatographic run time is not less than 2.5 times the retention time of the ciclopirox peak.] The relative retention times are about 0.5 for ciclopirox related compound A, 0.9 for 6-cyclohexyl-4-methyl-2(1*H*)-pyridone, 1.0 for ciclopirox, and 1.3 for ciclopirox related compound B. The peak response at 220 nm of the ciclopirox related compound A peak in the chromatogram obtained from the *Test solution* is not more than the peak response at 220 nm of the corresponding peak in the chromatogram obtained from *Standard solution A* (0.5% with reference to ciclopirox). The sum of responses at 298 nm of the impurity peaks in the chromatogram obtained from the *Test solution* is not more than the peak response at 298 nm of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution A* (0.5% with reference to ciclopirox). At 298 nm disregard any peak due to the solvent and any peak with a response less than the response of the ciclopirox related compound B peak in the chromatogram obtained from *Standard solution B* at 298 nm (0.1% with reference to ciclopirox).

**Assay**—Dissolve 200 mg of Ciclopirox Olamine, accurately weighed, in 2 mL of methanol. Add 38 mL of water, mix, and titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary corrections. Determine the factor of the 0.1 N sodium hydroxide VS using 100 mg of benzoic acid, accurately weighed, and titrate under the conditions prescribed above. Each mL of 0.1 N sodium hydroxide is equivalent to 26.84 mg of ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ).

## Ciclopirox Olamine Cream

» Ciclopirox Olamine Cream contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ).

**Packaging and storage**—Preserve in collapsible tubes, and store at controlled room temperature.

### USP Reference standards (11)—

USP Benzyl Alcohol RS

USP Ciclopirox Olamine RS

**Identification**—Dilute 4 mL of the *Assay preparation* obtained as directed in the *Assay* with a mixture of methanol and 6.25 N sodium hydroxide (123:2) to make 100 mL: the UV absorption spectrum of the solution so obtained exhibits maxima and minima at the same wavelengths as that of a

similar solution prepared from the *Standard preparation* obtained as directed in the *Assay*, concomitantly measured.

**Minimum fill (755):** meets the requirements.

**pH (791)**—Add 15 mL of boiling water, previously adjusted with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide to a pH of 6 to 7, to 3.5 g of Cream in a 50-mL centrifuge tube. Place a cap on the tube, and shake vigorously until an emulsion is formed. Loosen the cap, and heat the tube on a steam bath for 10 minutes. Allow to cool, centrifuge, and determine the pH of the aqueous phase: the pH is between 5.0 and 8.0.

### Content of benzyl alcohol (if present)—

**Solvent mixture**—Mix chloroform and methanol (4:1).

**Internal standard solution**—Prepare a solution of 1-nonyl alcohol in *Solvent mixture* containing about 1.75 mg per mL.

**Standard preparation**—Dilute an accurately weighed quantity of USP Benzyl Alcohol RS, quantitatively and stepwise, with *Solvent mixture* to obtain a solution having a known concentration of about 2 mg per mL. Transfer 5.0 mL of this solution and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, dilute with *Solvent mixture* to volume, and mix.

**Test preparation**—Transfer 1.0 g of Cream to a 50-mL volumetric flask, add about 30 mL of *Solvent mixture*, and mix. Add 5.0 mL of *Internal standard solution*, dilute with *Solvent mixture* to volume, and mix to obtain a clear solution.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and contains a 4-mm  $\times$  2-m glass column packed with 3% phase G3 on 100- to 120-mesh support S1AB. The column is maintained at a temperature of about 100°, the injection port and detector temperatures are maintained at about 315°, and nitrogen is used as the carrier gas at a flow rate of about 45 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the resolution,  $R_f$  between the peaks is not less than 1.6; the tailing factor for the benzyl alcohol peak and the internal standard peak is not greater than 3.5; and the relative standard deviation for replicate injections is not more than 3%.

**Procedure**—Separately inject equal volumes (about 2  $\mu$ L) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. [NOTE—After six injections, raise the column temperature to about 300° for about 5 minutes, then cool to 100°.] Calculate the percentage of benzyl alcohol in the Cream taken by the formula:

$$C(R_U / R_S),$$

in which C is the concentration, in mg per mL, of benzyl alcohol in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios of the benzyl alcohol peak to the internal standard peak obtained from the *Test preparation* and the *Standard preparation*, respectively: between 90.0% and 110.0% of the claimed amount is present.

### Assay—

**Ferrous sulfate solution**—Transfer 600 mg of ferrous sulfate to a 25-mL volumetric flask. Add 0.6 mL of glacial acetic acid, dilute with water to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Ciclopirox Olamine RS in methanol to obtain a solution having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Transfer an accurately weighed quantity of Cream, equivalent to about 10 mg of ciclopirox olamine, to a 50-mL volumetric flask, add 25 mL of methanol, and shake by mechanical means for about 10 minutes. Dilute with methanol to volume, mix, centrifuge, and use the supernatant.

**Procedure**—Transfer 4.0 mL of the *Standard preparation*, 4.0 mL of the *Assay preparation*, and 4.0 mL of methanol to



provide a blank, to separate 25-mL volumetric flasks. Add 15 mL of methanol to each flask, and mix. Then to each flask add 1.0 mL of *Ferrous sulfate solution*, mix, dilute with methanol to volume, and mix. Store the flasks in the dark for 1 hour. Concomitantly determine the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* against the blank in 1-cm cells at the wavelength of maximum absorbance at about 440 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ) in each g of the Cream taken by the formula:

$$50(C/W)(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Ciclopirox Olamine RS in the *Standard preparation*; W is the weight, in g, of Cream taken; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.

## Ciclopirox Olamine Topical Suspension

» Ciclopirox Olamine Topical Suspension contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ciclopirox olamine ( $C_{12}H_{17}NO_2 \cdot C_2H_7NO$ ).

**Packaging and storage**—Preserve in tight containers.

### USP Reference standards (11)—

USP Benzyl Alcohol RS

USP Ciclopirox Olamine RS

**Identification**—It responds to the *Identification* test under *Ciclopirox Olamine Cream*.

**Minimum fill** (755): meets the requirements.

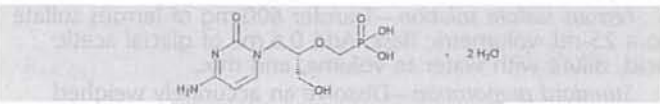
**pH** (791)—Proceed as directed for pH under *Ciclopirox Olamine Cream*, except to read "Topical Suspension" in place of "Cream" throughout.

**Content of benzyl alcohol**—Proceed as directed for *Content of benzyl alcohol* under *Ciclopirox Olamine Cream*, except to read "Topical Suspension" in place of "Cream" throughout.

**Assay**—Proceed as directed in the *Assay* under *Ciclopirox Olamine Cream*, except to read "Topical Suspension" in place of "Cream" throughout.

### Add the following:

## ▲Cidofovir



$C_8H_{14}N_3O_6P \cdot 2H_2O$  315.22

Phosphonic acid, [[2-(4-amino-2-oxo-1(2H)-pyrimidinyl)-1-(hydroxymethyl)ethoxy]methyl]-, dihydrate, (S)-;

1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]cytosine dihydrate [149394-66-1].

Anhydrous

$C_8H_{14}N_3O_6P$  279.19  
[113852-37-2].

## DEFINITION

Cidofovir contains NLT 98.0% and NMT 102.0% of cidofovir ( $C_8H_{14}N_3O_6P$ ), calculated on the anhydrous basis.

## IDENTIFICATION

### • A. INFRARED ABSORPTION (197K)

• B. The retention time of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### • PROCEDURE

[NOTE—Solutions containing cidofovir are stable at room temperature for 8 h.]

**Solution A:** Acetonitrile and water (40:60)

**Buffer:** Dissolve 1.2 g of dibasic ammonium phosphate and 2.0 g of tetrabutylammonium phosphate in 1 L of water. Adjust with ammonium hydroxide to a pH of 9.2.

**Mobile phase:** *Solution A* and *Buffer* (22:78)

**Standard solution:** 0.1 mg/mL of USP Cidofovir RS in water

**Sample solution:** 0.1 mg/mL of Cidofovir in water

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 274 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** 0.8–1.5

**Relative standard deviation:** NMT 0.73%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cidofovir ( $C_8H_{14}N_3O_6P$ ) in the portion of Cidofovir taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cidofovir RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cidofovir in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

## IMPURITIES

### • RESIDUE ON IGNITION (281)

**Analysis:** Perform the ignition at 850 ± 50°; silica crucibles are suitable.

**Acceptance criteria:** NMT 0.5%

### • ORGANIC IMPURITIES

[NOTE—Solutions containing cidofovir are stable at room temperature for 8 h.]

**Mobile phase and Chromatographic system:** Proceed as directed in the *Assay* with a run time four times the retention time of cidofovir.

**System suitability solution:** 1.5 μg/mL each of USP Cidofovir Related Compound A RS and USP Cidofovir Related Compound B RS in water

**Standard solution:** 0.001 mg/mL of USP Cidofovir RS in water

**Sample solution:** 1 mg/mL of Cidofovir in water

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.5 between cidofovir related compound A and cidofovir related compound B, *System suitability solution*



Relative standard deviation: NMT 5%, *Standard solution*

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Cidofovir taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of each individual impurity from the *Sample solution*

$r_s$  = peak response of cidofovir from the *Standard solution*

$C_s$  = concentration of USP Cidofovir RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Cidofovir in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 1*)

#### Acceptance criteria

Individual impurities: See *Table 1*. Disregard any impurity peaks less than 0.02%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cidofovir diol analog <sup>a</sup>	0.30	1.3	0.15
Cidofovir related compound A	0.54	0.74	0.15
Cidofovir related compound B	0.63	0.69	0.15
Cidofovir	1.0	—	—
Cidofovir uracil analog <sup>b</sup>	1.4	0.56	0.15
Bromocidofovir <sup>c</sup>	2.0	0.62	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

<sup>a</sup> 1-[(S)-2,3-Dihydroxypropyl]cytosine.

<sup>b</sup> 1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]uracil.

<sup>c</sup> 1-[(S)-3-Bromo-2-(phosphonomethoxy)propyl]cytosine.

#### • ENANTIOMERIC PURITY

Mobile phase: Dissolve 1.0 g of cupric sulfate in 1 L of water. Add 1.32 g of L-phenylalanine and sonicate to dissolve.

System suitability solution: 1 mg/mL of USP Cidofovir RS and 0.01 mg/mL of USP Cidofovir Enantiomer RS in water

Standard solution: 0.01 mg/mL of USP Cidofovir Enantiomer RS in water

Sample solution: 1 mg/mL of Cidofovir in water

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Temperatures

Autosampler: 15°

Column: 15°

Flow rate: 0.5 mL/min

Injection volume: 10 μL

Run time: Two times the retention time of cidofovir

#### System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—Typical relative retention times for cidofovir and cidofovir enantiomer are 1.0 and 1.3, respectively.]

#### Suitability requirements

Resolution: NLT 2.0 between cidofovir and cidofovir enantiomer, *System suitability solution*

Relative standard deviation: NMT 5%, *Standard solution*

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cidofovir enantiomer in the portion of Cidofovir taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of cidofovir enantiomer from the *Sample solution*

$r_s$  = peak response of cidofovir enantiomer from the *Standard solution*

$C_s$  = concentration of USP Cidofovir Enantiomer RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Cidofovir in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 1.0%

#### SPECIFIC TESTS

• **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic bacterial count is NMT  $10^2$  cfu/g. The total combined molds and yeasts count is NMT  $10^1$  cfu/g.

• **WATER DETERMINATION** (921), *Method I*, *Method Ia*

Sample: 0.2 g

Acceptance criteria: 10.5%–12.5%

• **PH** (791)

Sample solution: 1 g in 100 mL of water

Acceptance criteria: 2.5–4.5

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Cidofovir RS

USP Cidofovir Enantiomer RS

1-[(R)-3-Hydroxy-2-(phosphonomethoxy)propyl]cytosine dihydrate.

$C_8H_{14}N_3O_6P \cdot 2H_2O$  315.22

USP Cidofovir Related Compound A RS

1-[(S)-3-Hydroxy-2-(O-ethylphosphonomethoxy)propyl]cytosine.

$C_{10}H_{18}N_3O_6P$  307.24

USP Cidofovir Related Compound B RS

1-[(S)-3-Hydroxy-2-(O,O-diethylphosphonomethoxy)propyl]cytosine hydrochloride.

$C_{12}H_{22}N_3O_6P \cdot HCl$  371.76

▲ USP40

Add the following:

#### ▲ Cidofovir Injection

#### DEFINITION

Cidofovir Injection is a sterile aqueous solution. It contains an amount of cidofovir equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of anhydrous cidofovir ( $C_8H_{14}N_3O_6P$ ).

#### IDENTIFICATION

• **A. ULTRAVIOLET ABSORPTION** (197U)

Standard solution: 7.5 μg/mL of USP Cidofovir RS in water. Adjust with 0.1 N sodium hydroxide to a pH of 7.5.

Sample solution: Nominally 7.5 μg/mL of cidofovir from Injection in water



Acceptance criteria: Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### PROCEDURE

**Solution A:** Acetonitrile and water (40:60)

**Buffer:** Dissolve 1.2 g of dibasic ammonium phosphate and 2.0 g of tetrabutylammonium phosphate in 1 L of water. Adjust with ammonium hydroxide to a pH of 9.2.

**Mobile phase:** *Solution A* and *Buffer* (20:80)

**Standard solution:** 0.17 mg/mL of USP Cidofovir RS in water. [NOTE—0.17 mg/mL of USP Cidofovir RS is equivalent to 0.15 mg/mL of cidofovir on the anhydrous basis.]

**Sample solution:** Nominally 0.15 mg/mL of cidofovir from Injection in water

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 274 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Temperatures**

**Autosampler:** 10°

**Column:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 20 μL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anhydrous cidofovir ( $C_8H_{14}N_3O_6P$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of cidofovir from the *Sample solution*

$r_S$  = peak response of cidofovir from the *Standard solution*

$C_S$  = concentration of USP Cidofovir RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of anhydrous cidofovir in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of cidofovir (anhydrous), 279.19

$M_{r2}$  = molecular weight of cidofovir dihydrate, 315.22

**Acceptance criteria:** 95.0%–105.0% on the anhydrous basis

## IMPURITIES

### ORGANIC IMPURITIES

**Mobile phase and Chromatographic system:** Proceed as directed in the *Assay* with a run time NLT 4.5 times the retention time of cidofovir.

**Standard solution:** 0.0015 mg/mL of USP Cidofovir RS in water

**Sample solution:** Nominally 1.5 mg/mL of anhydrous cidofovir from Injection in water

## System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Relative standard deviation:** NMT 5.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of cidofovir from the *Standard solution*

$C_S$  = concentration of USP Cidofovir RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of anhydrous cidofovir in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of cidofovir (anhydrous), 279.19

$M_{r2}$  = molecular weight of cidofovir dihydrate, 315.22

### Acceptance criteria

**Individual impurities:** See *Table 1*.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cidofovir diol analog <sup>a,b</sup>	0.30	—	—
Cidofovir related compound A <sup>b</sup>	0.54	—	—
Cidofovir related compound B <sup>b</sup>	0.63	—	—
Cidofovir	1.0	—	—
Cidofovir uracil analog <sup>c</sup>	1.4	0.56	5.0
Bromocidofovir <sup>b,d</sup>	2.0	—	—
Any individual unspecified impurity	—	—	0.2
Total impurities	—	—	6.0

<sup>a</sup> 1-[(S)-2,3-Dihydroxypropyl]cytosine.

<sup>b</sup> These are included in the table for identification only. These are process impurities controlled in the drug substance. They are not to be included in the total impurities.

<sup>c</sup> 1-[(S)-3-Hydroxy-2-(phosphonomethoxy)propyl]uracil.

<sup>d</sup> 1-[(S)-3-Bromo-2-(phosphonomethoxy)propyl]cytosine.

## SPECIFIC TESTS

• **pH** <791>: 7.1–7.7

• **PARTICULATE MATTER IN INJECTIONS** <788>: Meets the requirements

• **BACTERIAL ENDOTOXINS TEST** <85>: NMT 1 USP Endotoxin Unit/mg of anhydrous cidofovir

• **STERILITY TESTS** <71>: Meets the requirements

• **OSMOLALITY AND OSMOLARITY** <785>, *Osmolality*: 550–650 mOsm/L

• **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* <1>

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose containers. Store at controlled room temperature.

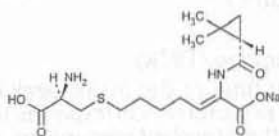
• **USP REFERENCE STANDARDS** <11>

USP Cidofovir RS  
USP Endotoxin RS

▲USP40



## Cilastatin Sodium



$C_{16}H_{25}N_2NaO_5S$  380.43

2-Heptenoic acid, 7-[[[(2-amino-2-carboxyethyl)thio]-2-[[[(2,2-dimethylcyclopropyl)carbonyl]amino]-, monosodium salt, [R-[R\*,S\*-(Z)]]-

Sodium (Z)-7-[[[(R)-2-amino-2-carboxyethyl]thio]-2-[(S)-2,2-dimethylcyclopropanecarboxamido]-2-heptenoate [81129-83-1].

» Cilastatin Sodium contains not less than 98.0 percent and not more than 101.5 percent of  $C_{16}H_{25}N_2NaO_5S$ , calculated on the anhydrous and solvent-free basis.

### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, *Packaging for constitution* (CN 1-May-2017), and store in a cold place.

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile.

### USP Reference standards (11)—

USP Cilastatin Ammonium Salt RS

USP Endotoxin RS

### Identification—

**A:** The retention time of the major peak for cilastatin in the chromatogram of the *Test solution*, as obtained in the test for *Chromatographic purity*, corresponds to that in the chromatogram of a similar preparation of USP Cilastatin Ammonium Salt RS.

**B:** Ignite a small portion of it on a platinum wire in a nonluminous flame: an intense yellow color is imparted to the flame.

**Specific rotation** (781S): between +41.5° and +44.5°, on the anhydrous and solvent-free basis.

*Test solution:* 10 mg per mL, in a mixture of methanol and hydrochloric acid (120:1).

**Bacterial Endotoxins Test** (85)—Where the label states that Cilastatin Sodium is sterile, it contains not more than 0.17 USP Endotoxin Unit per mg of cilastatin.

**Sterility Tests** (71)—Where the label states that Cilastatin Sodium is sterile, it meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined*, 6 g of specimen dissolved in 200 mL of *Fluid A* being used.

**pH** (791): between 6.5 and 7.5, in a solution (1 in 100).

**Water Determination, Method I** (921): not more than 2.0%.

### Delete the following:

• **Heavy metals, Method II** (231): 0.002%. (Official 1-Jan-2018)

### Limit of solvents—

*Internal standard solution*—Transfer 0.5 mL of *n*-propyl alcohol to a 1000-mL volumetric flask, dilute with water to volume, and mix.

*Standard solution*—Transfer 2.0 mL of acetone, 0.50 mL of methanol, and 0.50 mL of mesityl oxide to a 1000-mL volu-

metric flask, dilute with water to volume, and mix. Transfer 2.0 mL of this solution and 2.0 mL of *Internal standard solution* to a 10-mL volumetric flask, dilute with water to volume, and mix. This solution contains 316 µg of acetone, 79 µg of methanol, and 86 µg of mesityl oxide per mL.

*Test solution*—Transfer about 200 mg of Cilastatin Sodium, accurately weighed, to a 10-mL volumetric flask, add 2.0 mL of *Internal standard solution* and about 5 mL of water, and dissolve by shaking. Dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a flame-ionization detector and a 0.53-mm × 30-m capillary column, the internal wall of which is coated with a 1.0-µm film of liquid phase G16. The column temperature is maintained at 50° for 2.5 minutes, then increased at a rate of 8° per minute to 70°, and maintained at 70° for 0.5 minute; the injection port temperature is maintained at 160°; the detector temperature is maintained at 250°; and helium is used as the carrier gas at a flow rate of about 9 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.26 for acetone, 0.35 for methanol, 0.67 for *n*-propyl alcohol, and 1.0 for mesityl oxide; and the relative standard deviation for replicate injections, determined from peak area ratios of each analyte to *n*-propyl alcohol, is not more than 5.0%.

**Procedure**—Separately inject equal volumes (about 1 µL) of the *Standard solution* and the *Test solution* into the chromatograph, using the solvent (water) flush technique; record the chromatograms; and measure the areas for the acetone, methanol, *n*-propyl alcohol, and mesityl oxide peaks. Calculate the percentages of acetone, methanol, and mesityl oxide in the portion of Cilastatin Sodium taken by the formula:

$$(C/W)(R_U / R_S)$$

in which *C* is the concentration, in µg per mL, of the appropriate analyte in the *Standard solution*; *W* is the quantity, in mg, of Cilastatin Sodium taken to prepare the *Test solution*; and *R<sub>U</sub>* and *R<sub>S</sub>* are the peak area ratios of the corresponding analyte to *n*-propyl alcohol obtained from the *Test solution* and the *Standard solution*, respectively. Not more than 1.0% of acetone is found; not more than 0.5% of methanol is found; and not more than 0.4% of mesityl oxide is found.

### Chromatographic purity—

*Solvent*—Use water.

*Solution A*—Prepare a mixture of dilute phosphoric acid (1 in 1000) and acetonitrile (700:300), pass through a filter having a 0.5-µm or finer porosity, and degas.

*Solution B*—Use dilute phosphoric acid (1 in 1000). Pass through a filter having a 0.5-µm or finer porosity, and degas.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Test solution*—Prepare a solution of Cilastatin Sodium in *Solvent* having a concentration of about 1.6 mg per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.5-mm × 25-cm column containing packing L1. The column is maintained at a constant temperature of about 50°. The flow rate is about 2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0	15	85	equilibration
0–30	15→100	85→0	linear gradient



Chromatograph the *Test solution*, and measure the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is not less than 10; the column efficiency determined from the cilastatin peak is not less than 3000 theoretical plates; and the tailing factor is not more than 4.5.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Test solution* and the *Solvent* into the chromatograph, record the chromatograms, and measure the areas of the peaks. Calculate the chromatographic purity, in percentage, of the portion of Cilastatin Sodium taken by the formula:

$$100r_c / (r_T - r_B - r_A)$$

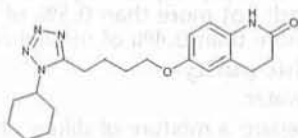
in which  $r_c$  is the area of the cilastatin peak obtained from the *Test solution*;  $r_T$  is the sum of the areas of all the peaks obtained from the *Test solution*;  $r_B$  is the sum of the areas of all the peaks obtained from the *Solvent*; and  $r_A$  is the response of the peak, if any, of nonretained substances, such as acetone, at the solvent front obtained from the *Test solution*: not less than 98.5% is found. Calculate the percentage of each impurity in the portion of Cilastatin Sodium taken by the formula:

$$100r_i / (r_T - r_B - r_A)$$

in which  $r_i$  is the peak area for each impurity in the chromatogram obtained from the *Test solution* and the other terms are as defined above: not more than 0.5% of any individual impurity is found.

**Assay**—Transfer about 300 mg of Cilastatin Sodium, accurately weighed, to a suitable beaker, add 30 mL of methanol, and dissolve by swirling. Add 5 mL of water, and titrate potentiometrically with 0.1 N hydrochloric acid to a pH of about 3. Then titrate with 0.1 N sodium hydroxide until three inflection points have been observed. Calculate the titer difference, in mL, between the first and third inflection points. Each mL of 0.1 N sodium hydroxide is equivalent to 19.022 mg of  $C_{16}H_{25}N_2NaO_5S$ .

## Cilostazol



$C_{20}H_{27}N_5O_2$  369.46

2-(1*H*)-Quinolinone, 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-

6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-carbostyryl [73963-72-1].

» Cilostazol contains not less than 98.0 percent and not more than 102.0 percent of  $C_{20}H_{27}N_5O_2$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, and store at room temperature.

**USP Reference standards** (11)—

USP Cilostazol RS

USP Cilostazol Related Compound A RS  
6-Hydroxy-3,4-dihydro-1*H*-quinolin-2-one.  
 $C_9H_9NO_2$  163.17

USP Cilostazol Related Compound B RS  
6-[4-(1-Cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-1*H*-quinolin-2-one.  
 $C_{20}H_{25}N_5O_2$  367.45

USP Cilostazol Related Compound C RS

1-(4-(5-Cyclohexyl-1*H*-tetrazol-1-yl)butyl)-6-(4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy)-3,4-dihydroquinolin-2(1*H*)-one.

$C_{31}H_{43}N_9O_3$  589.73

**Identification**—

**A: Infrared Absorption** (197K).

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Loss on drying** (731)—Dry it at 110° for 3 hours: it loses not more than 0.3% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Chloride** (221)—

**Test solution**—Dissolve 0.5 g of Cilostazol in 40 mL of dimethylformamide, add 6 mL of diluted nitric acid and dimethylformamide to make 50 mL.

**Control solution**—To 0.25 mL of 0.01 M hydrochloric acid add 6 mL of diluted nitric acid and dimethylformamide to make 50 mL.

**Procedure**—Add 1 mL of silver nitrate TS to the *Test solution* and to the *Control solution*, mix well, and allow to stand for 5 minutes, protecting from direct sunlight. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely. The opalescence developed in the *Test solution* is not more than that of the *Control solution* (0.018%).

**Delete the following:**

• **Heavy metals, Method II** (231): 0.001%. (Official 1-Jan-2018)

**Related compounds**—

**Diluent, Solution A, Solution B, Mobile phase, System suitability solution, and Chromatographic system**—Proceed as directed in the *Assay*.

**Standard solution**—Dissolve accurately weighed quantities of USP Cilostazol RS and USP Cilostazol Related Compound C RS in acetonitrile, with sonication if necessary, to obtain a solution having known concentrations of about 0.5 mg per mL of each component. Transfer 4 mL of this solution to a 10-mL volumetric flask, and dilute with water to volume. Further dilute this solution, stepwise if necessary, with *Diluent* to obtain a solution having known concentrations of about 0.4  $\mu$ g per mL of each component.

**Test solution**—Transfer about 20 mg of Cilostazol, accurately weighed, to a 50-mL volumetric flask, dissolve in 20 mL of acetonitrile, with sonication if necessary. Dilute with water to volume, and mix.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the percentage of cilostazol related compound C by the formula:

$$0.1(C_S / C_T) (r_U / r_S)$$

in which  $C_S$  is the concentration, in  $\mu$ g per mL, of cilostazol related compound C in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of Cilostazol in the *Test solution*;  $r_U$  is the peak response for cilostazol related compound C obtained from the *Test solution*; and  $r_S$  is the peak response for cilostazol related compound C obtained from the *Standard solution*. Calculate the percentage of other impurities by the formula:

$$0.1(1/F) (C_S / C_T) (r_U / r_S)$$

in which  $F$  is the relative response factor from Table 1;  $C_S$  is the concentration, in  $\mu$ g per mL, of cilostazol in the *Standard solution*;  $C_T$  is the concentration, in mg per mL, of cilostazol in the *Test solution*;  $r_U$  is the peak response for any



other impurity obtained from the *Test solution*; and  $r_s$  is the peak response for cilostazol obtained from the *Standard solution*.

Table 1

Name	Relative Retention Time	Relative Response Factor (F)	Limit (%)
Cilostazol related compound A <sup>1</sup>	0.2	1.7	0.1
Cilostazol related compound B <sup>2</sup>	0.9	0.58	0.1
Cilostazol	1.0	1.0	—
Cilostazol related compound C <sup>3</sup>	1.9	—	0.1
Any other individual impurity	—	1.0	0.1

<sup>1</sup>6-Hydroxy-3,4-dihydro-1H-quinolin-2-one

<sup>2</sup>6-[4-(1-Cyclohexyl-1H-tetrazol-5-yl)butoxy]-1H-quinolin-2-one

<sup>3</sup>1-(4-(5-Cyclohexyl-1H-tetrazol-1-yl)butyl)-6-(4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one

In addition to not exceeding the limits for impurities in Table 1, not more than 0.4% of total impurities is found.

#### Assay—

*Diluent*—Use a mixture of water and acetonitrile (60:40).

*Solution A*—Use a mixture of water and acetonitrile (70:30).

*Solution B*—Use a mixture of water and acetonitrile (50:50).

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

*System suitability solution*—Prepare a solution in *Diluent* having known concentrations of about 0.05 mg per mL each of USP Cilostazol RS, USP Cilostazol Related Compound A RS, and USP Cilostazol Related Compound B RS.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Cilostazol RS in acetonitrile, with sonication if necessary, to obtain a solution having a known concentration of about 1.0 mg per mL. Transfer 4 mL of this solution to a 10-mL volumetric flask, and dilute with water to volume. Further dilute this solution with *Diluent* to obtain a solution having a known concentration of about 0.04 mg per mL.

*Assay preparation*—Transfer about 20 mg of Cilostazol, accurately weighed, to a 50-mL volumetric flask, dissolve in 20 mL of acetonitrile, sonicate if necessary, dilute with water to volume, and mix. Transfer 1 mL of this solution to a 10-mL volumetric flask, dilute with *Diluent* to volume, and mix.

*Chromatographic system* (see *Chromatography* <621>)—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 10-cm column that contains 3.5-μm packing L7. The flow rate is about 1.0 mL per minute. The column temperature is maintained at 40°. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–6.5	100→50	0→50	linear gradient
6.5–10	50→0	50→100	linear gradient
10–20	0	100	isocratic
20–20.1	0→100	100→0	linear gradient
20.1–28	100	0	re-equilibration

Chromatograph the *System suitability solution*, identify the components using Table 1, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between cilostazol related compound B and cilostazol is not less than 3.0; the tailing factor for the cilostazol peak is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of C<sub>20</sub>H<sub>27</sub>N<sub>5</sub>O<sub>2</sub> in the portion of Cilostazol taken by the formula:

$$500C(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of cilostazol in the *Standard preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cilostazol Tablets

### DEFINITION

Cilostazol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cilostazol (C<sub>20</sub>H<sub>27</sub>N<sub>5</sub>O<sub>2</sub>).

### IDENTIFICATION

#### A. INFRARED ABSORPTION (197S)

*Standard solution*: 100 mg/mL of USP Cilostazol RS in chloroform

*Sample solution*: Transfer the equivalent of 100 mg of cilostazol from finely powdered Tablets into a glass container. Add 1 mL of chloroform, shake for 1 min, and pass through a suitable filter of 0.5-μm or finer pore size.

- B. The retention time of the cilostazol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

*Mobile phase*: Acetonitrile, methanol, and water (7:3:10)

*Internal standard solution*: 4 mg/mL of benzophenone in methanol

*Standard solution*: 0.1 mg/mL of USP Cilostazol RS and 0.04 mg/mL of *Internal standard solution* in methanol

*Sample solution*: Transfer the equivalent of 50 mg of cilostazol from powdered Tablets (NLT 20) into a suitable volumetric flask and add an appropriate quantity of *Internal standard solution*. Dilute with methanol to obtain a solution of 0.1 mg/mL of USP Cilostazol RS and 0.04 mg/mL of the internal standard. Pass a portion of this solution through a membrane filter of 0.5-μm or finer pore size, and use the filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

*Mode*: LC

*Detector*: UV 254 nm

*Column*: 4.6-mm × 15-cm; packing L1

*Flow rate*: 1 mL/min

*Injection size*: 10 μL

#### System suitability

*Sample*: *Standard solution*

#### Suitability requirements

*Resolution*: NLT 9.0 between the cilostazol and benzophenone peaks, eluted in this order



Relative standard deviation: NMT 1.5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of cilostazol ( $C_{20}H_{27}N_5O_2$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of cilostazol to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of cilostazol to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Cilostazol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cilostazol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

###### Test 1

**Medium:** 0.30% sodium lauryl sulfate in water; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 60 min

**Standard solution:** 0.28 mg/mL of USP Cilostazol RS in methanol. Dilute this solution with *Medium* to obtain a solution with a final concentration of about 5.6 µg/mL.

**Sample solution:** Pass NLT 20 mL of the solution under test through a suitable filter of 0.45-µm pore size, discarding the first 10 mL. Dilute with *Medium* to obtain a final theoretical concentration of about 5.6 µg/mL, considering complete dissolution of the label claim.

**Wavelength:** UV 257 nm

**Path length:** 1 cm

**Blank:** *Medium*

Calculate the percentage of cilostazol dissolved:

$$\text{Result} = (A_U/A_S) \times (C_S/L) \times V \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$L$  = Tablet label claim

$V$  = volume of *Medium*, 900 mL

**Tolerances:** NLT 80% (Q) of the labeled amount of cilostazol is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.3% sodium lauryl sulfate in water; 900 mL, deaerated

**Apparatus 2:** 75 rpm

**Time:** 30 min

**Standard solution:** Prepare a solution containing 1.1 mg/mL of USP Cilostazol RS in methanol. Dilute this solution with 0.5% sodium lauryl sulfate in water to obtain a final concentration of (L/900) mg/mL, where L is the Tablet label claim in mg.

**Sample solution:** Pass a portion of the solution under test through a suitable filter.

**Wavelength:** UV 258 nm

**Path length:** 0.2 cm

**Blank:** *Medium*

**Tolerances:** NLT 75% (Q) of the labeled amount of cilostazol is dissolved.

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

**Medium:** 0.3% sodium lauryl sulfate in water; 900 mL

**Apparatus 2:** 75 rpm

**Time:** 60 min

**Standard solution, Sample solution, Wavelength, Path length, and Blank:** Proceed as directed for *Test 1*.

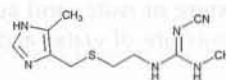
**Tolerances:** NLT 70% (Q) of the labeled amount of cilostazol is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight and light-resistant containers. Store at controlled room temperature.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**  
USP Cilostazol RS

## Cimetidine



$C_{10}H_{16}N_6S$

252.34

Guanidine, N'-cyano-N-methyl-N'-[2-[(5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]-; 2-Cyano-1-methyl-3-[2-[(5-methylimidazol-4-yl)methyl]thio]ethyl]guanidine [51481-61-9].

#### DEFINITION

Cimetidine contains NLT 98.0% and NMT 102.0% of cimetidine ( $C_{10}H_{16}N_6S$ ), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The UV absorption spectrum of a solution (1 in 80,000) in 0.1 N sulfuric acid exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Cimetidine RS, concomitantly measured.

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, and filter.

**Standard stock solution:** 0.4 mg/mL of USP Cimetidine RS in methanol and water (1:4), prepared by initially dissolving the USP Cimetidine RS in methanol using 20% of the final volume and diluting that solution with water to volume.

**Standard solution:** 0.01 mg/mL of USP Cimetidine RS in *Mobile phase* from the *Standard stock solution*.

**Sample stock solution:** 0.4 mg/mL of Cimetidine in methanol and water (1:4), prepared by initially dissolving the sample in methanol using 20% of the final volume and diluting that solution with water to volume.

**Sample solution:** 0.01 mg/mL of Cimetidine in *Mobile phase* from the *Sample stock solution*.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2.0 mL/min

Injection volume: 50 µL

**System suitability**Sample: *Standard solution***Suitability requirements**Capacity factor, *k'*: NLT 0.6

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of cimetidine ( $C_{10}H_{16}N_6S$ ) in the portion of Cimetidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Cimetidine RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Cimetidine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.2%

**Delete the following:**

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm • (Official 1-

(Jan-2018)

• **ORGANIC IMPURITIES**

**Mobile phase:** Mix 240 mL of methanol, 0.3 mL of 85% phosphoric acid, 940 mg of sodium 1-hexanesulfonate, and sufficient water to make 1 L, and filter.

**Standard solution:** 0.80 µg/mL of USP Cimetidine RS in *Mobile phase*

**Sample solution:** 0.4 mg/mL of Cimetidine in *Mobile phase*. Mix, sonicate for 15 min, and then mix again.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2.0 mL/min

Injection volume: 50 µL

**System suitability**Sample: *Standard solution***Suitability requirements**Capacity factor, *k'*: NLT 3.0

Column efficiency: NLT 2000 theoretical plates

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cimetidine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each impurity from the *Sample solution* $r_S$  = peak response of cimetidine from the *Standard solution* $C_S$  = concentration of USP Cimetidine RS in the *Standard solution* (µg/mL) $C_U$  = concentration of Cimetidine in the *Sample solution* (µg/mL)**Acceptance criteria**

Any individual impurity: NMT 0.2%

Total impurities: NMT 1.0%

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE** (741): 139°–144°

- **LOSS ON DRYING** (731)

Analysis: Dry a sample at 110° for 2 h.

Acceptance criteria: NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- **USP REFERENCE STANDARDS** (11)

USP Cimetidine RS

**Cimetidine Injection****DEFINITION**

Cimetidine Injection is a sterile solution of Cimetidine Hydrochloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cimetidine ( $C_{10}H_{16}N_6S$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, and filter.

**Standard stock solution:** 0.5 mg/mL of USP Cimetidine Hydrochloride RS in a mixture of methanol and water (1:4)

**Standard solution:** 12.5 µg/mL of USP Cimetidine Hydrochloride RS in *Mobile phase* from *Standard stock solution*

**Sample solution:** Nominally 10.0 µg/mL of cimetidine, prepared as follows. Transfer an accurately measured volume of Injection, equivalent to about 2 mg of cimetidine, to a 200-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 50 µL

**System suitability**Sample: *Standard solution***Suitability requirements**Capacity factor, *k'*: NLT 0.6

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of cimetidine ( $C_{10}H_{16}N_6S$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Cimetidine Hydrochloride RS in the *Standard solution* (mg/mL)



- $C_U$  = nominal concentration of cimetidine in the Sample solution (mg/mL)  
 $M_{r1}$  = molecular weight of cimetidine, 252.34  
 $M_{r2}$  = molecular weight of cimetidine hydrochloride, 288.81

Acceptance criteria: 90.0%–110.0%

### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.5 USP Endotoxin Unit/mg of cimetidine hydrochloride
- **PH (791):** 3.8–6.0
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products (1)*.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.
- **USP REFERENCE STANDARDS (11)**  
 USP Cimetidine Hydrochloride RS  
 USP Endotoxin RS

## Cimetidine Tablets

### DEFINITION

Cimetidine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cimetidine ( $C_{10}H_{16}N_6S$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Mobile phase:** Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, and filter.

**Standard stock solution:** 0.4 mg/mL of USP Cimetidine RS in methanol and water (1:4), prepared by initially dissolving the USP Cimetidine RS in methanol using 20% of the final volume and diluting that solution with water to volume

**Standard solution:** 0.01 mg/mL of USP Cimetidine RS in Mobile phase from the Standard stock solution

**Sample stock solution:** Weigh, and finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to about 100 mg of cimetidine, to a 250-mL volumetric flask. Add 50 mL of methanol, shake for 2 min, add 40 mL of water, sonicate for 15 min, and dilute with water to volume.

**Sample solution:** Transfer 5.0 mL of the Sample stock solution to a 200-mL volumetric flask, and dilute with Mobile phase to volume.

**Chromatographic system**  
 (See *Chromatography (621)*, *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2.0 mL/min

Injection volume: 50 µL

**System suitability**

Sample: Standard solution

**Suitability requirements**

Capacity factor,  $k'$ : NLT 0.6

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2.0%

**Analysis:** Calculate the percentage of the labeled amount of cimetidine ( $C_{10}H_{16}N_6S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the Sample solution  
 $r_S$  = peak response from the Standard solution  
 $C_S$  = concentration of USP Cimetidine RS in the Standard solution (mg/mL)  
 $C_U$  = nominal concentration of cimetidine in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0%

### PERFORMANCE TESTS

#### DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 1: 100 rpm. A 20-mesh basket may be used for 800-mg strength Tablets.

Time: 15 min

Standard solution: USP Cimetidine RS in Medium

Sample solution: Filtered solution under test, diluted with Medium to a concentration that is similar to that of the Standard solution

Detector: UV, the wavelength of maximum absorbance at about 218 nm

Tolerances: NLT 80% (Q) of the labeled amount of cimetidine ( $C_{10}H_{16}N_6S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
 USP Cimetidine RS

## Cimetidine in Sodium Chloride Injection

### DEFINITION

Cimetidine in Sodium Chloride Injection is a sterile solution of Cimetidine Hydrochloride and Sodium Chloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cimetidine ( $C_{10}H_{16}N_6S$ ) and NLT 95.0% and NMT 110.0% of the labeled amount of sodium chloride (NaCl).

### IDENTIFICATION

- **A.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.
- **B. IDENTIFICATION TESTS—GENERAL, Sodium (191) and Chloride (191):** Meets the requirements

### ASSAY

#### CIMETIDINE

**Mobile phase:** Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, and filter.

**Standard stock solution:** 0.5 mg/mL of USP Cimetidine Hydrochloride RS in a mixture of methanol and water (1:4)

**Standard solution:** 12.5 µg/mL of USP Cimetidine Hydrochloride RS in Mobile phase from Standard stock solution

**Sample solution:** Nominally 10.0 µg/mL of cimetidine, prepared as follows. Transfer an accurately measured volume of Injection, equivalent to about 2 mg of cimetidine, to a 200-mL volumetric flask, and dilute with Mobile phase to volume.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 50 µL

**System suitability**Sample: *Standard solution***Suitability requirements**Capacity factor, *k'*: NLT 0.6

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of cimetidine ( $C_{10}H_{16}N_6S$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Cimetidine Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of cimetidine in the *Sample solution* (mg/mL) $M_{r1}$  = molecular weight of cimetidine, 252.34 $M_{r2}$  = molecular weight of cimetidine hydrochloride, 288.81

Acceptance criteria: 90.0%–110.0%

**• SODIUM CHLORIDE****Sample solution:** Dilute a volume of Injection with water to obtain a solution containing 0.5 mg/mL of sodium chloride.**Analysis:** Determine the total amount of chloride, *A*, in the *Sample solution*, in mg, by titrating the *Sample solution* with 0.1 N silver nitrate VS, using a silver-silver chloride electrode. Each mL of 0.1 N silver nitrate is equivalent to 3.545 mg of chloride.To correct for the chloride present as cimetidine hydrochloride, calculate the concentration of chloride, *C*, due to sodium chloride, in mg/mL, in the *Sample solution*:

$$\text{Result} = (A/V) - [W \times (M_{Cl}/M_{r1})]$$

*A* = total amount of chloride in the *Sample solution* (mg)*V* = volume of the Injection taken to prepare the *Sample solution* (mL)*W* = quantity of cimetidine in the Injection, as determined in the *Assay for Cimetidine* (mg/mL) $M_{Cl}$  = atomic weight of chloride, 35.453 $M_{r1}$  = molecular weight of cimetidine, 252.34

Calculate the percentage of the labeled amount of sodium chloride (NaCl) in the portion of Injection taken:

$$\text{Result} = (C/C_U) \times (M_{NaCl}/M_{Cl}) \times 100$$

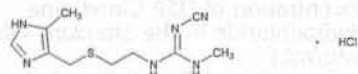
*C* = concentration of chloride due to sodium chloride in the *Sample solution* (mg/mL) $C_U$  = nominal concentration of sodium chloride in the *Sample solution* (mg/mL) $M_{NaCl}$  = molecular weight of sodium chloride, 58.443 $M_{Cl}$  = atomic weight of chloride, 35.453

Acceptance criteria: 95.0%–110.0%

**SPECIFIC TESTS**• **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.5 USP Endotoxin Unit/mg of cimetidine hydrochloride• **pH (791):** 5.0–7.0• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in single-dose glass or plastic containers. Glass containers are preferably of Type I or Type II glass.• **USP REFERENCE STANDARDS (11)**

USP Cimetidine Hydrochloride RS

USP Endotoxin RS

**Cimetidine Hydrochloride**

$C_{10}H_{16}N_6S \cdot HCl$  288.80  
 Guanidine, *N*''-cyano-*N*-methyl-*N*'-[2-[[[5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]-, monohydrochloride;  
 2-Cyano-1-methyl-3-[2-[[[5-methylimidazol-4-yl)methyl]thio]ethyl]guanidine monohydrochloride  
 [70059-30-2].

**DEFINITION**Cimetidine Hydrochloride contains NLT 98.0% and NMT 102.0% of cimetidine hydrochloride ( $C_{10}H_{16}N_6S \cdot HCl$ ), calculated on the dried basis.**IDENTIFICATION**• **A. INFRARED ABSORPTION (197K)**• **B. ULTRAVIOLET ABSORPTION (197U)**

Sample solution: 14 µg/mL

Medium: 0.1 N sulfuric acid

Acceptance criteria: Meets the requirements

**ASSAY****• PROCEDURE****Mobile phase:** Transfer 200 mL of methanol and 0.3 mL of phosphoric acid to a 1000-mL volumetric flask, dilute with water to volume, and filter.**Standard stock solution:** 0.5 mg/mL of USP Cimetidine Hydrochloride RS in a mixture of methanol and water (1:4)**Standard solution:** 12.5 µg/mL of USP Cimetidine Hydrochloride RS in *Mobile phase* from *Standard stock solution***Sample stock solution:** 0.5 mg/mL of Cimetidine Hydrochloride in a mixture of methanol and water, prepared by initially dissolving the sample in water using 20% of the final volume, adding methanol using 20% of the final volume, and diluting that solution with water to volume**Sample solution:** 12.5 µg/mL of Cimetidine Hydrochloride in *Mobile phase* from *Sample stock solution*



**Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 50 µL

**System suitability**

Sample: Standard solution

**Suitability requirements**Capacity factor,  $k'$ : NLT 0.6

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2.0%

**Analysis**

Samples: Standard solution and Sample solution

Calculate the percentage of cimetidine hydrochloride ( $C_{10}H_{16}N_6S \cdot HCl$ ) in the portion of Cimetidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the Sample solution $r_S$  = peak response from the Standard solution $C_S$  = concentration of USP Cimetidine Hydrochloride in the Standard solution (mg/mL) $C_U$  = concentration of Cimetidine Hydrochloride in the Sample solution (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 0.2%

**Delete the following:**

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-

Jan-2018)

- **ORGANIC IMPURITIES**

**Mobile phase:** Transfer 940 mg of sodium 1-hexanesulfonate to a 1000-mL volumetric flask, add 240 mL of methanol followed by 0.3 mL of phosphoric acid, and dilute with water to volume. Filter before use.

**Sample solution:** 0.4 mg/mL of Cimetidine Hydrochloride in Mobile phase

**Diluted sample solution:** 0.8 µg/mL of Cimetidine Hydrochloride in Mobile phase from the the Sample solution

**System suitability solution:** Dissolve 50 mg of Cimetidine Hydrochloride in 10 mL of 1 N hydrochloric acid, heat on a steam bath for about 10 min (or boil on a hot plate for about 2 min), and allow to cool. Dilute a suitable volume of this solution with Mobile phase to obtain a solution containing 2 µg/mL. Use this solution within 24 h of its preparation. Adjustment of the heating step may be necessary to achieve a satisfactory amide analog peak response for the measurement of the resolution between the cimetidine and the amide analog peaks.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 2 mL/min

Injection volume: 50 µL

**System suitability**

Samples: System suitability solution and Diluted sample solution

**Suitability requirements**

Resolution: NLT 4.0 between the cimetidine and the amide analog peaks, System suitability solution

Capacity factor,  $k'$ : NLT 3.0, Diluted sample solution

Column efficiency: NLT 2000 theoretical plates, Diluted sample solution

Relative standard deviation: NMT 7.0%, Diluted sample solution

**Analysis**

Samples: Sample solution and Diluted sample solution  
Calculate the percentage of each impurity in the portion of Cimetidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times D \times 100$$

 $r_U$  = peak response for each impurity from the Sample solution $r_S$  = peak response of cimetidine from the Diluted sample solution $D$  = dilution factor to prepare the Diluted sample solution from the Sample solution, 0.002**Acceptance criteria**

Any individual impurity: NMT 0.2%

Total impurities: NMT 1.0%

**SPECIFIC TESTS**

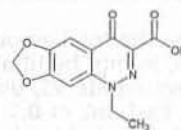
- **LOSS ON DRYING (731)**

Analysis: Dry a sample at 105° for 2 h.

Acceptance criteria: NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Cimetidine Hydrochloride RS

**Cinoxacin**

$C_{12}H_{10}N_2O_5$  262.22  
[1,3]Dioxolo[4,5-g]cinnoline-3-carboxylic acid, 1-ethyl-1,4-dihydro-4-oxo-;  
1-Ethyl-1,4-dihydro-4-oxo[1,3]dioxolo[4,5-g]cinnoline-3-carboxylic acid [28657-80-9].

**DEFINITION**Cinoxacin contains NLT 97.0% and NMT 102.0% of cinoxacin ( $C_{12}H_{10}N_2O_5$ ), calculated on the dried basis.**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B.** The  $R_f$  value of the principal spot of the Sample solution corresponds to that of Standard solution A, as obtained in the test for Organic Impurities.

**ASSAY**

- **PROCEDURE**

**Solution A:** 38.1 mg/mL of sodium borate in water**Internal standard solution:** An aqueous solution containing 2 mg/mL of sulfanilic acid and 5.0 mL of Solution A in each 100 mL**Mobile phase:** Dilute 100.0 mL of Solution A and 0.426 g of sodium sulfate with water to 1000 mL. The quantity of sodium sulfate may be varied to meet System suitability requirements and to provide a suitable elution time.**Standard stock solution:** 1 mg/mL of USP Cinoxacin RS in Solution A**Standard solution:** 50 µg/mL of USP Cinoxacin RS prepared as follows. Dilute 5.0 mL of Standard stock solution and 5.0 mL of Internal standard solution with water to 100 mL.



**Sample stock solution:** 1 mg/mL of Cinoxacin in *Solution A*

**Sample solution:** Dilute 5.0 mL of *Sample stock solution* and 5.0 mL of *Internal standard solution* with water to 100 mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 1.8-mm × 1-m; packing L12

**Flow rate:** 1 mL/min

**Injection volume:** 1.0 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for cinoxacin and sulfanilic acid are 1.0 and 2.2, respectively.]

#### Suitability requirements

**Resolution:** NLT 4.4 between cinoxacin and sulfanilic acid

**Tailing factor:** NMT 2.1 for cinoxacin

**Relative standard deviation:** NMT 2.0% from five replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cinoxacin ( $C_{12}H_{10}N_2O_5$ ) in the portion of Cinoxacin taken:

$$\text{Result} = (R_u/R_s) \times (C_s/C_u) \times 100$$

$R_u$  = peak response ratio of cinoxacin to sulfanilic acid from the *Sample solution*

$R_s$  = peak response ratio of cinoxacin to sulfanilic acid from the *Standard solution*

$C_s$  = concentration of USP Cinoxacin RS in the *Standard solution* (µg/mL)

$C_u$  = concentration of Cinoxacin in the *Sample solution* (µg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Diluent:** Chloroform, dimethylformamide, dimethyl sulfide, and nitromethane (1:1:1:1)

**Standard solution A:** 5 mg/mL of USP Cinoxacin RS in *Diluent*

**Standard solution B:** 0.05 mg/mL from *Standard solution A* in *Diluent*

**Sample solution:** 5 mg/mL of Cinoxacin in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10 µL

**Developing solvent system:** Acetonitrile, ammonium hydroxide, and water (105: 7.5: 30)

#### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

In a suitable chromatographic chamber lined with paper, place a volume of the *Developing solvent system* sufficient to develop the chromatogram, cover, and allow to equilibrate for 30 min. Apply the *Samples*, dry the plate, and apply each sample three additional times at the corresponding initial locations. Dry the plate thoroughly after each application, and develop the chromatogram until the solvent front has moved to the top of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. View the plate under short- and long-wavelength UV light.

**Acceptance criteria:** 1.0%; the  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of *Standard solution A*, and no spot of the *Sample solution* other than the principal spot is larger or more intense than the principal spot of *Standard solution B*.

#### SPECIFIC TESTS

##### • LOSS ON DRYING (731)

**Analysis:** Dry a sample under vacuum at 60° for 3 h.

**Acceptance criteria:** NMT 1.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Cinoxacin RS

## Cinoxacin Capsules

#### DEFINITION

Cinoxacin Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of cinoxacin ( $C_{12}H_{10}N_2O_5$ ).

#### IDENTIFICATION

##### • A. THIN-LAYER CHROMATOGRAPHY

**Diluent:** Chloroform, dimethylformamide, dimethyl sulfide, and nitromethane (1:1:1:1)

**Standard solution:** 5 mg/mL of USP Cinoxacin RS in *Diluent*

**Sample solution:** Equivalent to 5 mg/mL of cinoxacin from Capsules in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10 µL

**Developing solvent system:** Acetonitrile, ammonium hydroxide, and water (105: 7.5: 30)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

In a suitable chromatographic chamber lined with paper, place a volume of the *Developing solvent system* sufficient to develop the chromatogram, cover, and allow to equilibrate for 30 min. Apply the *Samples*, dry the plate, and apply each sample three additional times at the corresponding initial locations. Dry the plate thoroughly after each application, and develop the chromatogram until the solvent front has moved to the top of the plate. Remove the plate from the chamber, and allow the solvent to evaporate. View the plate under short- and long-wavelength UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

#### ASSAY

##### • PROCEDURE

**Sample solution:** Nominally 10 µg/mL prepared as follows. Transfer an equivalent to 250 mg of cinoxacin to a 100-mL volumetric solution from the contents of NLT 20 Capsules. Dilute with 0.1 M sodium borate to volume. Filter the solution, discarding the first 20 mL of the filtrate, and dilute with water to 10 µg/mL.

**Standard stock solution:** 2.5 mg/mL of USP Cinoxacin RS in 0.1 M sodium borate

**Standard solution:** 10 µg/mL of USP Cinoxacin RS in water, prepared from *Standard stock solution*



**Instrumental conditions**

Mode: UV

Analytical wavelength: 352 nm

Cell: 1 cm

Blank: 0.1 M sodium borate and water (2 in 500)

**Analysis**Samples: *Sample solution* and *Standard solution*Calculate the percentage of the labeled amount of cinoxacin ( $C_{12}H_{10}N_2O_3$ ) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

 $A_U$  = absorbance of the *Sample solution* $A_S$  = absorbance of the *Standard solution* $C_S$  = concentration of the *Standard solution* ( $\mu\text{g/mL}$ ) $C_U$  = nominal concentration of the *Sample solution* ( $\mu\text{g/mL}$ )

Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**• **DISSOLUTION (711)**Buffer: pH 6.5 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*)

Medium: Buffer, 500 mL for Capsules containing 250 mg or less of cinoxacin; 1000 mL for Capsules containing more than 250 mg of cinoxacin

Apparatus 1: 100 rpm

Time: 30 min

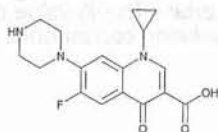
Standard solution: 0.35 mg/mL of USP Cinoxacin RS in Medium

Sample solutions: Filter, and dilute with 0.1 N sodium hydroxide solution as needed.

**Instrumental conditions**

Mode: UV

Analytical wavelength: 270 nm

**Analysis**Samples: *Standard solution* and *Sample solution*Tolerances: NLT 60% (Q) of the labeled amount of cinoxacin ( $C_{12}H_{10}N_2O_3$ ) is dissolved.• **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers.• **USP REFERENCE STANDARDS (11)**  
USP Cinoxacin RS**Ciprofloxacin** $C_{17}H_{18}FN_3O_3$ 

331.34

3-Quinolonecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-;

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolonecarboxylic acid [85721-33-1].

**DEFINITION**Ciprofloxacin contains NLT 98.0% and NMT 102.0% of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ), calculated on the dried basis.**IDENTIFICATION**• **A. INFRARED ABSORPTION (197K)**• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.**ASSAY**• **PROCEDURE**Buffer: 0.025 M phosphoric acid. Adjust with triethylamine to a pH of  $3.0 \pm 0.1$ .

Mobile phase: Acetonitrile and Buffer (13:87)

Standard solution: 0.5 mg/mL of USP Ciprofloxacin RS prepared as follows. Transfer 12.5 mg of USP Ciprofloxacin RS to a 25-mL volumetric flask. Add 0.1 mL of 7% phosphoric acid, and dilute with *Mobile phase* to volume.System suitability stock solution: 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*System suitability solution: Transfer 1.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.Sample solution: Transfer 25 mg of Ciprofloxacin to a 50-mL volumetric flask. Add 0.2 mL of 7% phosphoric acid, and dilute with *Mobile phase* to volume.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm  $\times$  25-cm; packing L1Column temperature:  $30 \pm 1^\circ$ 

Flow rate: 1.5 mL/min

Injection volume: 10  $\mu\text{L}$ **System suitability**Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are about 0.7 and 1.0, respectively.]

**Suitability requirements**Resolution: NLT 6 between ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*Tailing factor: NMT 2.5, *Standard solution*Relative standard deviation: NMT 1.5%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ) in the portion of Ciprofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak area from the *Sample solution* $r_S$  = peak area from the *Standard solution* $C_S$  = concentration of USP Ciprofloxacin RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Ciprofloxacin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

**IMPURITIES**• **RESIDUE ON IGNITION (281):** NMT 0.1%, except that where it is intended for use in preparing Ciprofloxacin for Oral Suspension, it is NMT 0.2%.**Delete the following:**• **HEAVY METALS (231), Method II:** NMT 20 ppm • (Official 1: Jan-2018)• **ORGANIC IMPURITIES**Buffer: Dilute 3.4 mL of phosphoric acid with water to 2000 mL. Adjust with triethylamine to a pH of  $3.0 \pm 0.1$ .



**Solution A:** Acetonitrile  
**Mobile phase:** See Table 1.

Table 1

Time (min)	Buffer (%)	Solution A (%)
0	87	13
10	87	13
11	50	50
16	50	50
16.1	87	13
20	87	13

**Diluent:** Solution A and Buffer (13:87)

**System suitability solution:** 7.5 µg/mL each of USP Ciprofloxacin Ethylenediamine Analog RS and USP Ciprofloxacin RS in Diluent

**Standard stock solution:** 0.1 mg/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin RS prepared as follows. Add suitable amounts of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin RS to a suitable volumetric flask. Add 0.1% of the flask volume of 6 M ammonium hydroxide and dilute with water to volume.

**Standard solution:** 0.7 µg/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin RS from Standard stock solution in Diluent

**Sample solution:** 0.35 mg/mL of Ciprofloxacin prepared as follows. Transfer 35 mg of Ciprofloxacin to a 100-mL volumetric flask, add 0.2 mL of 7% phosphoric acid, and dilute with Diluent to volume.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 263 and 278 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 30 µL

#### System suitability

**Samples:** System suitability solution and Standard solution

[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 6.0 between ciprofloxacin ethylenediamine analog and ciprofloxacin at 278 nm, System suitability solution

**Tailing factor:** NMT 2.0 for the ciprofloxacin peak at 278 nm, Standard solution

**Relative standard deviation:** NMT 5.0% for ciprofloxacin at 278 nm; NMT 5.0% for fluoroquinolonic acid at 263 nm, Standard solution

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of fluoroquinolonic acid in the portion of Ciprofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of fluoroquinolonic acid at 263 nm from the Sample solution  
 $r_S$  = peak response of fluoroquinolonic acid at 263 nm from the Standard solution  
 $C_S$  = concentration of USP Fluoroquinolonic Acid RS in the Standard solution (mg/mL)  
 $C_U$  = concentration of Ciprofloxacin in the Sample solution (mg/mL)

Calculate the percentage of the ciprofloxacin ethylenediamine analog and any individual unspecified impurity in the portion of Ciprofloxacin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of each impurity at 278 nm from the Sample solution  
 $r_S$  = peak response of ciprofloxacin at 278 nm from the Standard solution  
 $C_S$  = concentration of USP Ciprofloxacin RS in the Standard solution (mg/mL)  
 $C_U$  = concentration of Ciprofloxacin in the Sample solution (mg/mL)

**Acceptance criteria:** See Table 2. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Wavelength (nm)	Acceptance Criteria, NMT (%)
Ciprofloxacin ethylenediamine analog	0.70	278	0.2
Ciprofloxacin	1.00	278	—
Fluoroquinolonic acid	1.89	263	0.2
Any individual unspecified impurity	—	278	0.2
Total impurities <sup>a</sup>	—	—	0.5

<sup>a</sup> Total impurities does not include the fluoroquinolonic acid impurity.

#### SPECIFIC TESTS

##### • CLARITY OF SOLUTION

**Sample solution:** Dissolve 0.25 g in 10 mL of 0.1 N hydrochloric acid.

**Acceptance criteria:** A clear to slightly opalescent solution is obtained.

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): Where it is intended for use in preparing Ciprofloxacin for Oral Suspension, the total microbial count does not exceed 10<sup>3</sup> cfu/g, and the total combined molds and yeasts count does not exceed 10<sup>2</sup> cfu/g. It also meets the requirements for absence of *Salmonella* species and *Escherichia coli*.

##### • LOSS ON DRYING (731)

**Analysis:** Dry under vacuum at 120° for 6 h.

**Acceptance criteria:** NMT 1.0%, except that where it is labeled as intended for use in preparing Ciprofloxacin for Oral Suspension, 10%–20%

- **STERILITY TESTS** (71), *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*: Where the label states that it is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that it is sterile or where the label states that Ciprofloxacin must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.50 USP Endotoxin Units/mg of ciprofloxacin.

#### ADDITIONAL REQUIREMENTS

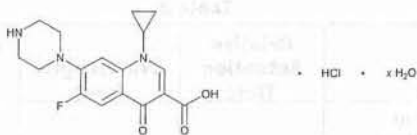
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°, and avoid excessive heat.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Where it is intended for use in preparing Ciprofloxacin for Oral Suspension, it is so labeled.



### • USP REFERENCE STANDARDS (11)

- USP Ciprofloxacin RS  
 USP Ciprofloxacin Ethylenediamine Analog RS  
 7-(2-Aminoethylamino)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.  
 $C_{15}H_{16}FN_3O_3$  305.30  
 USP Endotoxin RS  
 USP Fluoroquinolonic Acid RS  
 7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.  
 $C_{13}H_9ClFN_3O_3$  281.67

## Ciprofloxacin Hydrochloride



- $C_{17}H_{18}FN_3O_3 \cdot HCl \cdot xH_2O$   
 Sesquihydrate 394.83  
 Monohydrate 385.82  
 Anhydrous 367.81  
 3-Quinolincarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-, monohydrochloride;  
 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolincarboxylic acid, monohydrochloride  
 Monohydrate [86393-32-0].

### DEFINITION

Ciprofloxacin Hydrochloride contains NLT 98.0% and NMT 102.0% of ciprofloxacin hydrochloride ( $C_{17}H_{18}FN_3O_3 \cdot HCl$ ), calculated on the anhydrous basis. It contains a variable quantity of water.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **C. IDENTIFICATION TESTS—GENERAL** (191), Chloride

### ASSAY

#### • PROCEDURE

**Buffer:** 0.025 M phosphoric acid. Adjust with triethylamine to a pH of  $3.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Buffer* (13:87)

**Standard solution:** 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*

**System suitability stock solution:** 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*

**System suitability solution:** Transfer 1.0 mL of the *System suitability stock solution* to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.

**Sample solution:** 0.5 mg/mL of Ciprofloxacin Hydrochloride in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 278 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L1

**Column temperature:**  $30 \pm 1^\circ$

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 6 between ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

**Tailing factor:** NMT 2.5, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of ciprofloxacin hydrochloride ( $C_{17}H_{18}FN_3O_3 \cdot HCl$ ) in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Ciprofloxacin Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

### Delete the following:

- **HEAVY METALS** (231), *Method II*: NMT 20 ppm (Official 1-

Jan-2018)

- **ORGANIC IMPURITIES**

**Buffer:** Dilute 3.4 mL of phosphoric acid with water to 2000 mL. Adjust with triethylamine to a pH of  $3.0 \pm 0.1$ .

**Solution A:** Acetonitrile

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Buffer (%)	Solution A (%)
0	87	13
10	87	13
11	50	50
16	50	50
16.1	87	13
20	87	13

**Diluent:** *Solution A* and *Buffer* (13:87)

**System suitability solution:** 7.5  $\mu$ g/mL each of USP Ciprofloxacin Ethylenediamine Analog RS and USP Ciprofloxacin Hydrochloride RS in *Diluent*

**Standard stock solution:** 0.1 mg/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin Hydrochloride RS prepared as follows. Add suitable amounts of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin Hydrochloride RS to a suitable volumetric flask. Add 0.1% of the flask volume of 6 M ammonium hydroxide and dilute with water to volume.

**Standard solution:** 0.7  $\mu$ g/mL each of USP Fluoroquinolonic Acid RS and USP Ciprofloxacin Hydrochloride RS from *Standard stock solution* in *Diluent*

**Sample solution:** 0.35 mg/mL of Ciprofloxacin Hydrochloride in *Diluent*



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 263 and 278 nm**Column:** 4.6-mm × 25-cm; 5-μm packing L1**Column temperature:** 40°**Flow rate:** 1.5 mL/min**Injection volume:** 30 μL**System suitability****Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

**Suitability requirements****Resolution:** NLT 6.0 between ciprofloxacin ethylenediamine analog and ciprofloxacin at 278 nm, *System suitability solution***Tailing factor:** NMT 2.0 for the ciprofloxacin peak at 278 nm, *Standard solution***Relative standard deviation:** NMT 5.0% for ciprofloxacin at 278 nm; NMT 5.0% for fluoroquinolonic acid at 263 nm, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of fluoroquinolonic acid in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of fluoroquinolonic acid at 263 nm from the *Sample solution* $r_S$  = peak response of fluoroquinolonic acid at 263 nm from the *Standard solution* $C_S$  = concentration of USP Fluoroquinolonic Acid RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Ciprofloxacin Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of ciprofloxacin ethylenediamine analog and any individual unspecified impurity in the portion of Ciprofloxacin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each impurity at 278 nm from the *Sample solution* $r_S$  = peak response of ciprofloxacin at 278 nm from the *Standard solution* $C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Ciprofloxacin Hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** See Table 2. Disregard peaks less than 0.05%.**Table 2**

Name	Relative Retention Time	Wavelength (nm)	Acceptance Criteria, NMT (%)
Ciprofloxacin ethylenediamine analog	0.70	278	0.2
Ciprofloxacin	1.00	278	—

\* Total impurities does not include fluoroquinolonic acid impurity.

**Table 2 (Continued)**

Name	Relative Retention Time	Wavelength (nm)	Acceptance Criteria, NMT (%)
Fluoroquinolonic acid	1.89	263	0.2
Any individual unspecified impurity	—	278	0.2
Total impurities*	—	—	0.5

\* Total impurities does not include fluoroquinolonic acid impurity.

**SPECIFIC TESTS****• pH (791)****Sample solution:** 25-mg/mL solution in water**Acceptance criteria:** 3.0–4.5**• WATER DETERMINATION (921), Method I:** 4.7%–6.7%**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.**• USP REFERENCE STANDARDS (11)**

USP Ciprofloxacin Ethylenediamine Analog RS

7-(2-Aminoethylamino)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

 $C_{15}H_{18}FN_3O_3$  305.30

USP Ciprofloxacin Hydrochloride RS

USP Fluoroquinolonic Acid RS

7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

 $C_{13}H_9ClFN_3O_3$  281.67**Ciprofloxacin Injection****DEFINITION**Ciprofloxacin Injection is a sterile solution of Ciprofloxacin or Ciprofloxacin Hydrochloride in Water for Injection, in 5% Dextrose Injection, or in 0.9% Sodium Chloride Injection prepared with the aid of Lactic Acid. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ).**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE****Solution A:** 0.025 M phosphoric acid. Adjust with triethylamine to a pH of 3.0 ± 0.1.**Mobile phase:** Acetonitrile and *Solution A* (13:87)**Standard solution:** 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase***System suitability solution:** 0.025 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*. Transfer 1.0 mL of this solution to a 10-mL volumetric flask, and dilute with *Standard solution* to volume.**Sample solution:** Equivalent to 0.5 mg/mL of Ciprofloxacin from Injection diluted with *Mobile phase*



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm × 25-cm; packing L1

Temperature: 30 ± 1°

Flow rate: 1.5 mL/min

Injection size: 10 µL

**System suitability**Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

**Suitability requirements**

Resolution: NLT 6 between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak

Column efficiency: NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*Tailing factor: NMT 2.5 for the ciprofloxacin peak, *Standard solution*Relative standard deviation: NMT 1.5%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of C<sub>17</sub>H<sub>18</sub>FN<sub>3</sub>O<sub>3</sub> from the portion of Ciprofloxacin Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL) $M_{r1}$  = molecular weight of ciprofloxacin, 331.34 $M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

Acceptance criteria: 90.0%–110.0%

**OTHER COMPONENTS****• LACTIC ACID CONTENT**

Mobile phase: Acetonitrile and 0.005 N sulfuric acid (3:17)

Standard solution: 0.8 mg/mL of USP Sodium Lactate RS in water or 4 mg/mL where the Injection is labeled as being a concentrated form

Sample solution: Use the undiluted Injection.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 208 nm

Column: 7.8-mm × 30-cm; packing L17

Temperature: 40 ± 1°

Flow rate: 0.6 mL/min

Injection size: 20 µL

**System suitability**Sample: *Standard solution***Suitability requirements**

Tailing factor: NMT 2.0 for the analyte peak

Relative standard deviation: NMT 2.0%

[NOTE—After each analysis, rinse the column with a mixture of 0.01 N sulfuric acid and acetonitrile to elute the ciprofloxacin from the column. Promptly regenerate the column with 0.01 N sulfuric acid, and the column may be reused or stored.]

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the concentration of lactic acid (C<sub>3</sub>H<sub>6</sub>O<sub>3</sub>) in mg/mg of ciprofloxacin:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2})$$

 $r_U$  = peak response of lactic acid from the *Sample solution* $r_S$  = peak response of lactic acid from the *Standard solution* $C_S$  = concentration of USP Sodium Lactate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL) $M_{r1}$  = molecular weight of lactic acid, 90.08 $M_{r2}$  = molecular weight of sodium lactate, 112.07

Acceptance criteria: 0.288–0.352 mg of lactic acid for each mg of ciprofloxacin claimed on the label, except that where the Injection is labeled as being a concentrated form, it contains 0.335–0.409 mg of lactic acid for each mg of ciprofloxacin claimed on the label

**• DEXTROSE CONTENT (if present)**

Sample solution: Undiluted Injection

Analysis: Determine the angular rotation in a suitable polarimeter tube (see *Optical Rotation* (781)). Calculate the percentage (g/100 mL) of dextrose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> · H<sub>2</sub>O) in the portion of Injection taken:

$$\text{Result} = A \times R \times (M_{r1}/M_{r2}) \times (100/F)$$

 $A$  = 100 mm divided by the length of the polarimeter tube (mm) $R$  = observed rotation (degrees) $M_{r1}$  = molecular weight of dextrose monohydrate, 198.17 $M_{r2}$  = molecular weight of anhydrous dextrose, 180.16 $F$  = midpoint of the specific rotation range for anhydrous dextrose, 52.9°

Acceptance criteria: 4.75–5.25 g/100 mL

**• SODIUM CHLORIDE CONTENT (if present)**

Sample solution: Injection

Analysis: Transfer 10.0 mL of *Sample solution* to a suitable container, dilute with water to 150 mL, add 1.5 mL of potassium chromate TS, and titrate with 0.1 N silver nitrate TS. Each mL of 0.1 N silver nitrate is equivalent to 5.844 mg of sodium chloride (NaCl).

Acceptance criteria: 85.5–94.5 mg

**IMPURITIES****Organic Impurities****• PROCEDURE: LIMIT OF CIPROFLOXACIN ETHYLENEDIAMINE ANALOG**Mobile phase, *System suitability solution*, *Sample solution*, *Chromatographic system*, and *System suitability*: Proceed as directed in the *Assay*.**Analysis**Sample: *Sample solution*

Calculate the percentage of ciprofloxacin ethylenediamine analog from the portion of Ciprofloxacin Injection taken:

$$\text{Result} = [F \times r_A / (F \times r_A + r_C)] \times 100$$

 $F$  = correction factor for ciprofloxacin ethylenediamine analog, 0.7 $r_A$  = ciprofloxacin ethylenediamine analog peak response $r_C$  = peak response of ciprofloxacin

Acceptance criteria: NMT 0.5%

**SPECIFIC TESTS**• **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements• **pH** (791): 3.5–4.6, except that where the Injection is labeled as being a concentrated form, its pH is 3.3–3.9• **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.50 USP Endotoxin Unit/mg of ciprofloxacin.• **STERILITY TESTS** (71): It meets the requirements for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.



- **COLOR AND ACHROMICITY** (631) (where it is labeled as being a concentrated form): It has no more color than a solution prepared by diluting 5.0 mL of *Matching Fluid O* with 95.0 mL of 0.12 N hydrochloric acid.
- **OTHER REQUIREMENTS:** It meets the requirements for *Container Content for Injections* (697).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I glass, and store in a cool place or at controlled room temperature. Avoid freezing and exposure to light.
- **LABELING:** The label indicates whether the vehicle is Sterile Water for Injection, 5% Dextrose Injection, or 0.9% Sodium Chloride Injection. Label the Injection that has Sterile Water for Injection as the vehicle to indicate that it is a concentrated form that must be diluted to appropriate strength (1–2 mg/mL) with 5% Dextrose Injection or 0.9% Sodium Chloride Injection before administration, and that the resulting solution is stable for up to 14 days when stored in a cool place or at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)
  - USP Ciprofloxacin Ethylenediamine Analog RS
  - 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminophenyl)amino]-3-quinolinecarboxylic acid hydrochloride.
  - $C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77
  - USP Ciprofloxacin Hydrochloride RS
  - USP Endotoxin RS
  - USP Sodium Lactate RS

### Ciprofloxacin Ophthalmic Ointment

#### DEFINITION

Ciprofloxacin Ophthalmic Ointment contains an amount of Ciprofloxacin Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ).

#### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Buffer:** 1.7 g/L of tetrabutylammonium phosphate in water. Adjust with phosphoric acid to a pH of 2.0.  
**Mobile phase:** Methanol and *Buffer* (250:750)  
**Standard solution:** 0.033 mg/mL of USP Ciprofloxacin Hydrochloride RS in 0.1 N hydrochloric acid  
**System suitability solution:** 5 µg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Standard solution*  
**Sample solution:** Transfer an amount nominally equivalent to 750 µg of ciprofloxacin from Ophthalmic Ointment to a screw-capped tube. Add 15 mL of solvent hexane, and shake vigorously until the Ophthalmic Ointment is dispersed. Loosen the cap, and heat in a water bath at 60° for 30 min, with occasional swirling. Remove from the bath, tighten the cap, and shake for 1.5 min while still hot. Add 25.0 mL of 0.1 N hydrochloric acid, and shake vigorously for 1.5 min. Allow the layers to separate, and use the lower, aqueous layer.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for the ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.8 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

**Tailing factor:** 0.9–2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ) in the portion of Ophthalmic Ointment taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of ciprofloxacin, 331.34

$M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

**Acceptance criteria:** 90.0%–110.0%

#### SPECIFIC TESTS

- **STERILITY TESTS** (71): It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.
- **OTHER REQUIREMENTS:** It meets the requirements in *Ophthalmic Products—Quality Tests* (771).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible ophthalmic ointment tubes. Store at a temperature between 2° and 25°.
- **USP REFERENCE STANDARDS** (11)
  - USP Ciprofloxacin Ethylenediamine Analog RS
  - 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminophenyl)amino]-3-quinolinecarboxylic acid hydrochloride.
  - $C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77
  - USP Ciprofloxacin Hydrochloride RS

### Ciprofloxacin Ophthalmic Solution

#### DEFINITION

Ciprofloxacin Ophthalmic Solution is a sterile, aqueous solution of Ciprofloxacin Hydrochloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ).



**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****PROCEDURE**

**Solution A:** 0.005 M tetrabutylammonium phosphate solution. Adjust with phosphoric acid to a pH of 2.0.

**Mobile phase:** Methanol and *Solution A* (1:3)

**Standard solution:** 0.14 mg/mL of USP Ciprofloxacin Hydrochloride RS in water

**System suitability solution:** 0.01 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Standard solution*

**Sample solution:** Equivalent to 0.12 mg/mL of ciprofloxacin from Ophthalmic Solution, in water

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 20 µL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for the ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.8 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between the ciprofloxacin ethylenediamine analog and ciprofloxacin, *System suitability solution*

**Capacity factor:** 1.5–6 for the ciprofloxacin peak, *Standard solution*

**Column efficiency:** NLT 500 theoretical plates, *Standard solution*

**Tailing factor:** 0.9–2.0, *Standard solution*

**Relative standard deviation:** NMT 2%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of  $C_{17}H_{18}FN_3O_3$  in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of ciprofloxacin, 331.34

$M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- pH (791):** 3.5–5.5

- STERILITY TESTS (71):** It meets the requirements when tested as directed under *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers, protected from light, at room temperature.

**USP REFERENCE STANDARDS (11)**

USP Ciprofloxacin Ethylenediamine Analog RS

1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.

$C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77

USP Ciprofloxacin Hydrochloride RS

**Ciprofloxacin Tablets****DEFINITION**

Ciprofloxacin Tablets contain Ciprofloxacin Hydrochloride equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ).

**IDENTIFICATION**

The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****PROCEDURE**

**Solution A:** 0.025 M phosphoric acid. Adjust with triethylamine to a pH of  $2.0 \pm 0.1$ .

**Solution B:** Acetonitrile and *Solution A* (13:87)

**Solution C:** 0.025 M phosphoric acid. Adjust with triethylamine to a pH of  $3.0 \pm 0.1$ .

**Mobile phase:** Acetonitrile and *Solution C* (13:87)

**Standard solution:** 0.2 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Solution B*

**System suitability solution:** 0.05 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in the *Standard solution*

**Sample solution:** Transfer 5 Tablets to a 500-mL volumetric flask, add 400 mL of *Solution B*, and sonicate for about 20 min. Dilute with *Solution B* to volume, mix, and pass through a membrane filter of 0.45-µm pore size. Prepare the equivalent of 0.20 mg/mL of ciprofloxacin from the filtrate with *Solution B*.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 278 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Column temperature:**  $30 \pm 1^\circ$

**Flow rate:** 1.5 mL/min

**Injection size:** 10 µL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The retention time for ciprofloxacin is 6.4–10.8 min. The relative retention times for ciprofloxacin ethylenediamine analog and ciprofloxacin are 0.7 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 6 between the ciprofloxacin ethylenediamine analog peak and the ciprofloxacin peak, *System suitability requirements*

**Column efficiency:** NLT 2500 theoretical plates from the ciprofloxacin peak, *Standard solution*

**Tailing factor:** NMT 2.0 for the ciprofloxacin peak, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{17}H_{18}FN_3O_3$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response from the *Sample solution*



- $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL), calculated on the anhydrous basis  
 $C_u$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)  
 $M_{r1}$  = molecular weight of ciprofloxacin, 331.34  
 $M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid; 900 mL

Apparatus 2: 50 rpm

Time: 30 min

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

**Standard solution:** USP Ciprofloxacin Hydrochloride RS in *Medium*

### Spectrometric conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

Mode: UV

Analytical wavelength: 276 nm

### Analysis

**Samples:** *Sample solution* and *Standard solution*

**Tolerances:** An amount of ciprofloxacin hydrochloride ( $C_{17}H_{18}FN_3O_3 \cdot HCl$ ) equivalent to NLT 80% (Q) of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ) is dissolved.

### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE:

Preserve in well-closed containers.

### • USP REFERENCE STANDARDS (11)

USP Ciprofloxacin Ethylenediamine Analog RS  
 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminooethyl)amino]-3-quinolinecarboxylic acid hydrochloride.

$C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77

USP Ciprofloxacin Hydrochloride RS

## Ciprofloxacin Extended-Release Tablets

### DEFINITION

Ciprofloxacin Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Buffer:** Dilute 2.9 mL of phosphoric acid in water to 1000 mL. Adjust with triethylamine to a pH of 3.0.

**Mobile phase:** Acetonitrile and *Buffer* (135:865)

**System suitability solution:** 0.58 mg/mL of USP Ciprofloxacin Hydrochloride RS and 0.5 mg/mL of USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*

**Standard stock solution:** 1.16 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*

**Standard solution:** 0.058 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase* from *Standard stock solution*

**Sample stock solution:** Nominally 0.5 mg/mL in *Mobile phase* prepared as follows. Transfer an equivalent to 250 mg of ciprofloxacin from finely powdered Tablets (NLT 20) to a 500-mL volumetric flask. Add 400 mL of *Mobile phase*, place on a rotary shaker for 15 min, and sonicate for 25 min. Allow the solution to cool to room temperature, and dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45- $\mu$ m pore size.

**Sample solution:** Nominally 0.05 mg/mL of ciprofloxacin in water from *Sample stock solution*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 278 nm

Column: 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

Column temperature: 30°

Flow rate: 1.5 mL/min

Injection volume: 10  $\mu$ L

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 6 between the ciprofloxacin and ciprofloxacin ethylenediamine analog peaks, *System suitability solution*

**Tailing factor:** NMT 4.0 for the ciprofloxacin peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0% for the ciprofloxacin peak, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

$r_u$  = peak response of ciprofloxacin from the *Sample solution*

$r_s$  = peak response of ciprofloxacin from the *Standard solution*

$C_s$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of ciprofloxacin, 331.34

$M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

#### Test 1

**Medium:** pH 4.5 acetate buffer (transfer 3 g of sodium acetate and 14 mL of 2 N acetic acid to a 1-L volumetric flask, and dilute with water to volume); 900 mL, deaerated

**Apparatus 2:** 50 rpm

**Times:** 30, 60, and 120 min

**Standard solution:** 6.5  $\mu$ g/mL of USP Ciprofloxacin Hydrochloride RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45- $\mu$ m pore size. For 500-mg Tablets, transfer 2 mL of the filtrate to a 200-mL volumetric flask, and dilute with *Medium* to volume. For 1000-mg Tablets, transfer 1 mL of the filtrate to a 200-mL volumetric flask, and dilute with *Medium* to volume. Replace the aliquots withdrawn for analysis with fresh portions of *Medium*.



**Instrumental conditions**

Mode: UV

Analytical wavelength: 277 nm

Blank: Medium

**Analysis****Samples:** Standard solution and Sample solution

Calculate the percentage of ciprofloxacin

(C<sub>17</sub>H<sub>18</sub>FN<sub>3</sub>O<sub>3</sub>) dissolved at each time interval (D<sub>i</sub>):

$$D_i = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times D \times 100$$

A<sub>U</sub> = absorbance of the Sample solutionA<sub>S</sub> = absorbance of the Standard solutionC<sub>S</sub> = concentration of ciprofloxacin hydrochloride in the Standard solution (mg/mL)

L = label claim (mg/Tablet)

M<sub>r1</sub> = molecular weight of ciprofloxacin, 331.34M<sub>r2</sub> = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

V = volume of Medium, 900 mL

D = dilution factor of the Sample solution

Percentage of ciprofloxacin dissolved at the first time interval = D<sub>1</sub>Percentage of ciprofloxacin dissolved at the second time interval = D<sub>2</sub> + [D<sub>1</sub> × (v/V)]Percentage of ciprofloxacin dissolved at the third time interval = D<sub>3</sub> + [(D<sub>2</sub> + D<sub>1</sub>) × (v/V)]

v = volume of solution under test removed at each time interval (mL)

**Tolerances**

For Tablets labeled to contain 500 mg, see Table 1.

**Table 1**

Time (min)	Amount Dissolved
30	42%–62%
60	62%–87%
120	NLT 80%

For Tablets labeled to contain 1000 mg, see Table 2.

**Table 2**

Time (min)	Amount Dissolved
30	30%–50%
60	50%–70%
120	NLT 80%

The percentages of the labeled amount of ciprofloxacin (C<sub>17</sub>H<sub>18</sub>FN<sub>3</sub>O<sub>3</sub>) dissolved at the times specified conform to Acceptance Table 2 in Dissolution (711).

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Times:** 30, 60, and 120 min

**Standard solution:** 0.62 mg/mL of USP Ciprofloxacin Hydrochloride RS in Medium

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with Medium to a concentration similar to the Standard solution, if necessary.

**Instrumental conditions**

Mode: UV

Analytical wavelength: 276 nm

Cell length: 0.1 mm

Blank: Medium

**Analysis****Samples:** Standard solution and Sample solution

Calculate the percentage of ciprofloxacin

(C<sub>17</sub>H<sub>18</sub>FN<sub>3</sub>O<sub>3</sub>) dissolved at each time interval (D<sub>i</sub>):

$$D_i = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times D \times 100$$

A<sub>U</sub> = absorbance of the Sample solutionA<sub>S</sub> = absorbance of the Standard solutionC<sub>S</sub> = concentration of ciprofloxacin hydrochloride in the Standard solution (mg/mL)

L = label claim (mg/Tablet)

M<sub>r1</sub> = molecular weight of ciprofloxacin, 331.34M<sub>r2</sub> = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

V = volume of Medium, 900 mL

D = dilution factor of the Sample solution

Percentage of ciprofloxacin dissolved at the first time interval = D<sub>1</sub>Percentage of ciprofloxacin dissolved at the second time interval = D<sub>2</sub> + [D<sub>1</sub> × (v/V)]Percentage of ciprofloxacin dissolved at the third time interval = D<sub>3</sub> + [(D<sub>2</sub> + D<sub>1</sub>) × (v/V)]

v = volume of solution under test removed at each time interval (mL)

**Tolerances:** See Table 3.**Table 3**

Time Point (i)	Time (min)	Amount Dissolved
1	30	40%–65%
2	60	NLT 60%
3	120	NLT 80%

The percentages of the labeled amount of ciprofloxacin (C<sub>17</sub>H<sub>18</sub>FN<sub>3</sub>O<sub>3</sub>) dissolved at the times specified conform to Acceptance Table 2 in Dissolution (711).

**Test 3:** If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 3.

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Times:** 30, 60, and 120 min

**Standard solution:** 0.65 mg/mL of USP Ciprofloxacin Hydrochloride RS in Medium

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with Medium to a concentration similar to the Standard solution, if necessary.

**Instrumental conditions**

Mode: UV

Analytical wavelength: 350 nm

Cell length

For 500-mg Tablet strength: 2 mm

For 1000-mg Tablet strength: 1 mm

Blank: Medium

**Analysis****Samples:** Standard solution and Sample solution

Calculate the percentage of ciprofloxacin

(C<sub>17</sub>H<sub>18</sub>FN<sub>3</sub>O<sub>3</sub>) dissolved at each time interval (D<sub>i</sub>):

$$D_i = (A_U/A_S) \times (C_S/L) \times (M_{r1}/M_{r2}) \times V \times D \times 100$$

A<sub>U</sub> = absorbance of the Sample solutionA<sub>S</sub> = absorbance of the Standard solutionC<sub>S</sub> = concentration of USP Ciprofloxacin Hydrochloride RS in the Standard solution (mg/mL)

L = label claim (mg/Tablet)

M<sub>r1</sub> = molecular weight of ciprofloxacin, 331.34



$M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81  
 $V$  = volume of *Medium*, 900 mL  
 $D$  = dilution factor of the *Sample solution*  
 Percentage of ciprofloxacin dissolved at the first time interval =  $D_1$   
 Percentage of ciprofloxacin dissolved at the second time interval =  $D_2 + [D_1 \times (v/V)]$   
 Percentage of ciprofloxacin dissolved at the third time interval =  $D_3 + [(D_2 + D_1) \times (v/V)]$   
 $v$  = volume of solution under test removed at each time interval (mL)  
 Tolerances: See Table 4.

Table 4

Time Point (h)	Time (min)	Amount Dissolved
1	30	37%–57%
2	60	55%–75%
3	120	NLT 80%

The percentages of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ) dissolved at the times specified conform to Acceptance Table 2 in *Dissolution* (711).

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Buffer, Mobile phase, and System suitability solution:** Prepare as directed in the *Assay*.

**Standard stock solution:** 0.5 mg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase*

**Standard solution:** 1.25 µg/mL of USP Ciprofloxacin Hydrochloride RS in *Mobile phase* from *Standard stock solution*

**Sample solution:** Nominally 0.5 mg/mL of ciprofloxacin in *Mobile phase* prepared as follows. Transfer an equivalent to 250 mg of ciprofloxacin from finely powdered Tablets (NLT 20) to a 500-mL volumetric flask. Add 400 mL of *Mobile phase*, place on a rotary shaker for 15 min, and sonicate for 25 min with intermittent shaking. Allow the solution to cool to room temperature, and dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter of 0.45-µm pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 263 and 278 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 6 between the ciprofloxacin and ciprofloxacin ethylenediamine analog peaks at 278 nm, *System suitability solution*

**Tailing factor:** NMT 2.0 for the ciprofloxacin peak at 278 nm, *Standard solution*

**Relative standard deviation:** NMT 10.0% for the ciprofloxacin peak at 278 nm, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of decarboxyciprofloxacin in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

$r_U$  = peak response of decarboxyciprofloxacin at 263 nm from the *Sample solution*

$r_S$  = peak response of ciprofloxacin at 263 nm from the *Standard solution*

$C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of ciprofloxacin, 331.34

$M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

$F$  = relative response factor of decarboxyciprofloxacin (see Table 5)

Calculate the percentage of the other impurities in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity at 278 nm from the *Sample solution*

$r_S$  = peak response of ciprofloxacin at 278 nm from the *Standard solution*

$C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of ciprofloxacin, 331.34

$M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

$F$  = relative response factor (see Table 5)

Acceptance criteria: See Table 5.

Table 5

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Decarboxyciprofloxacin <sup>a</sup>	0.36	1.6	0.2
Desfluorociprofloxacin <sup>b</sup>	0.59	1.0	0.2
Ciprofloxacin ethylenediamine analog <sup>c</sup>	0.68	1.2	0.2
Ciprofloxacin	1.00	—	—
7-Chloro-6-piperazinyl analog <sup>d</sup>	1.20	0.45	0.2
Chlorociprofloxacin <sup>e</sup>	2.10	0.75	0.2
Any unspecified impurity	—	1.0	0.2
Total impurities	—	—	0.6

<sup>a</sup> 1-Cyclopropyl-6-fluoro-7-(piperazin-1-yl)quinolin-4(1H)-one.

<sup>b</sup> 1-Cyclopropyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

<sup>c</sup> 7-(2-Aminoethylamino)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

<sup>d</sup> 7-Chloro-1-cyclopropyl-4-oxo-6-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

<sup>e</sup> 6-Chloro-1-cyclopropyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if Test 1 is not used.



• **USP REFERENCE STANDARDS (11)**

USP Ciprofloxacin Ethylenediamine Analog RS  
7-(2-Aminoethylamino)-1-cyclopropyl-6-fluoro-4-oxo-  
1,4-dihydroquinoline-3-carboxylic acid.  
 $C_{15}H_{16}FN_3O_3$  305.30  
USP Ciprofloxacin Hydrochloride RS

## Ciprofloxacin and Dexamethasone Otic Suspension

### DEFINITION

Ciprofloxacin and Dexamethasone Otic Suspension is a sterile, aqueous suspension containing ciprofloxacin hydrochloride and dexamethasone. It contains NLT 90.0% and NMT 110.0% of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ) and NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone ( $C_{22}H_{29}FO_5$ ).

### IDENTIFICATION

- **A.** The *Sample solution*, obtained as directed in the *Assay* for *Ciprofloxacin*, exhibits a major peak for ciprofloxacin, the retention time of which corresponds to that of the *Standard solution*, obtained as directed in the *Assay* for *Ciprofloxacin*.
- **B.** The *Sample solution*, obtained as directed in the *Assay* for *Dexamethasone*, exhibits a major peak for dexamethasone, the retention time of which corresponds to that of the *Standard solution*, obtained as directed in the *Assay* for *Dexamethasone*.

### ASSAY

#### • CIPROFLOXACIN

**Buffer:** Add 6.0 mL of phosphoric acid and 8 g of diethylamine phosphate to 2.0 L of water. Adjust with 50% sodium hydroxide to a pH of 3.0.

**Mobile phase:** Acetonitrile and *Buffer* (11:89)

**System suitability solution:** 1.6 µg/mL each of USP Ciprofloxacin Hydrochloride RS and USP Ciprofloxacin Ethylenediamine Analog RS in *Mobile phase*

**Standard solution A:** 1.48 mg/mL of USP Ciprofloxacin Hydrochloride RS in 0.1 N hydrochloric acid. Dilute with *Mobile phase* to 0.13 mg/mL of ciprofloxacin.

**Standard solution B:** 0.0025 mg/mL of ciprofloxacin from *Standard solution A* in *Mobile phase*

**Sample solution:** Nominally 0.12 mg/mL in *Mobile phase* prepared as follows. Transfer the equivalent to 3 mg of ciprofloxacin from freshly mixed Otic Suspension to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm × 15-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution*, *Standard solution A*, and *Standard solution B*

#### Suitability requirements

**Resolution:** NLT 3.0 between ciprofloxacin and the ciprofloxacin ethylenediamine analog, *System suitability solution*

**Column efficiency:** NLT 2500 theoretical plates for ciprofloxacin, *System suitability solution*

**Tailing factor:** NMT 2.0 for ciprofloxacin, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution A* and *Standard solution B*

### Analysis

**Samples:** *Standard solution A* and *Sample solution*  
Calculate the percentage of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ ) in the portion of Otic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of ciprofloxacin from the *Sample solution*

$r_S$  = peak response of ciprofloxacin from *Standard solution A*

$C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in *Standard solution A* (mg/mL)

$C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of ciprofloxacin, 331.34

$M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of ciprofloxacin ( $C_{17}H_{18}FN_3O_3$ )

#### • DEXAMETHASONE

**Buffer and Mobile phase:** Prepare as directed in the test for *Limit of Ciprofloxacin Formamide*.

**Standard stock solution:** 2 mg/mL of USP Dexamethasone RS in acetonitrile

**System suitability solution:** 0.2 mg/mL of USP Dexamethasone RS and 0.2 mg/mL of USP Dexamethasone Acetate RS in *Mobile phase*

**Standard solution A:** 0.2 mg/mL of USP Dexamethasone RS from *Standard stock solution* in *Mobile phase*

**Standard solution B:** 0.004 mg/mL of USP Dexamethasone RS from *Standard solution A* in *Mobile phase*

**Sample solution:** Nominally 0.2 mg/mL in *Mobile phase* prepared as follows. Transfer freshly mixed Otic Suspension equivalent to 2 mg of dexamethasone to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 15-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 50 µL

#### System suitability

**Samples:** *System suitability solution*, *Standard solution A*, and *Standard solution B*

#### Suitability requirements

**Resolution:** NLT 12 between dexamethasone and dexamethasone acetate, *System suitability solution*

**Column efficiency:** NLT 2000 theoretical plates for dexamethasone, *System suitability solution*

**Tailing factor:** NMT 2.0 for dexamethasone, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution A* and *Standard solution B*

### Analysis

**Samples:** *Standard solution A* and *Sample solution*  
Calculate the percentage of the labeled amount of dexamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Otic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of dexamethasone from the *Sample solution*

$r_S$  = peak response of dexamethasone from *Standard solution A*

$C_S$  = concentration of USP Dexamethasone RS in *Standard solution A* (mg/mL)

$C_U$  = nominal concentration of dexamethasone in the *Sample solution* (mg/mL)



**Acceptance criteria:** 90.0%–110.0% of the labeled amount of dexamethasone ( $C_{22}H_{29}FO_5$ )

## IMPURITIES

### • LIMIT OF CIPROFLOXACIN FORMAMIDE

**Buffer:** Phosphoric acid and water (3:997). Adjust with 50% sodium hydroxide to a pH of 3.0.

**Mobile phase:** Acetonitrile and Buffer (27:73)

**Standard stock solution:** 0.25 mg/mL of USP Ciprofloxacin Formamide RS in methanol

**System suitability solution:** 0.025 mg/mL of USP Dexamethasone RS and 0.025 mg/mL of USP Ciprofloxacin Formamide RS prepared as follows. Transfer 2.5 mg of USP Dexamethasone RS and 2.5 mg of USP Ciprofloxacin Formamide RS in a 100-mL volumetric flask, and dissolve in 15 mL of methanol before diluting with *Mobile phase* to volume.

**Standard solution:** 0.015 mg/mL from *Standard stock solution* in *Mobile phase*

**Sample solution:** Nominally 0.6 mg/mL in *Mobile phase* prepared as follows. Transfer an amount of freshly mixed Otic Suspension, equivalent to 6 mg, to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm × 15-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 50 µL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 8 between ciprofloxacin formamide and dexamethasone, *System suitability solution*

**Column efficiency:** NLT 2000 theoretical plates for ciprofloxacin formamide, *Standard solution*

**Tailing factor:** NMT 2.0 for ciprofloxacin formamide, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each related compound in the portion of Otic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of ciprofloxacin formamide from the *Sample solution*

$r_S$  = peak response of ciprofloxacin formamide from the *Standard solution*

$C_S$  = concentration of USP Ciprofloxacin Formamide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of ciprofloxacin in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 0.5% of the labeled amount of ciprofloxacin

### • CIPROFLOXACIN RELATED COMPOUNDS

**Analysis:** From the chromatogram of the *Sample solution*, obtained as directed in the *Assay for Ciprofloxacin*, measure the responses for the ciprofloxacin ethylenediamine analog and the other minor peaks. Calculate the percentage of each related compound in the portion of Otic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (100/F)$$

$r_U$  = peak response of each related compound from the *Sample solution*

$r_S$  = peak response of ciprofloxacin from *Standard solution B*

$C_S$  = concentration of USP Ciprofloxacin Hydrochloride RS in *Standard solution B*, calculated on the anhydrous basis (mg/mL)

$C_U$  = nominal concentration of ciprofloxacin in the Otic Suspension (mg/mL)

$M_{r1}$  = molecular weight of ciprofloxacin, 331.34

$M_{r2}$  = molecular weight of anhydrous ciprofloxacin hydrochloride, 367.81

$F$  = relative response factor (1.3 for ciprofloxacin ethylenediamine analog and 1.0 assumed for all other degradation products)

### Acceptance criteria

**Ciprofloxacin ethylenediamine analog:** NMT 0.4% of the labeled amount of ciprofloxacin

**Other single related compound:** NMT 0.2%

**Sum of all related compounds:** NMT 0.8%

### • DEXAMETHASONE RELATED COMPOUNDS

**Analysis:** From the chromatogram of the *Sample solution*, obtained as directed in the *Assay for Dexamethasone*, measure the responses for the dexamethasone glyoxal analog, the 17-carboxy-17-deoxy analog, and other minor peaks. Calculate the percentage of each related compound in the portion of Otic Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each related compound from the *Sample solution*

$r_S$  = peak response of dexamethasone from *Standard solution B*

$C_S$  = concentration of USP Dexamethasone RS in *Standard solution B* (mg/mL)

$C_U$  = nominal concentration of dexamethasone in the Otic Suspension (mg/mL)

### Acceptance criteria

**Dexamethasone glyoxal analog:** NMT 1.0%

**17-Carboxy-17-deoxy analog:** NMT 2.6%

**Other single related compound:** NMT 0.3%

**Sum of all related compounds:** NMT 3.5%

[NOTE—The relative retention times for the dexamethasone glyoxal analog (9-Fluoro-11β-hydroxy-16α-methylandrosta-1,4-diene-3-one-17-ylglyoxal) and the 17-carboxy-17-deoxy analog (9-fluoro-11β-hydroxy-16α-methylandrosta-1,4-diene-3-one-17β-carboxylic acid) are about 1.4–1.6 and about 2.8–3.2, respectively.]

### SPECIFIC TESTS

#### • pH (791): 3.8–4.8

• **STERILITY TESTS (71):** It meets the requirements when tested as directed under *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.

#### • PARTICLE SIZE

**Carrier fluid:** Heat Purified Water to a temperature of 40°–50°, add 100 mg/L of dexamethasone while stirring, cool to room temperature while stirring, pass through a 0.2-µm filter, and store in a clean, covered container.

**Sample solution:** Dilute a volume of 10 µL of Otic Suspension with *Carrier fluid* to 25 mL.

### Analysis

(See *Particulate Matter in Injections* <788>, *Light Obscuration Particle Count Test*.)

Analyze the *Sample solution* using an electronic, liquid-borne particle counting system that employs a light obscuration sensor with a suitable sample feeding device.

**Acceptance criteria:** NLT 99.5% of the particles are ≤25 µm, NLT 99.95% are ≤50 µm, and NLT 99.995% are ≤100 µm.

• **OSMOLALITY AND OSMOLARITY (785), Osmolality:** 270–330 mOsm/kg

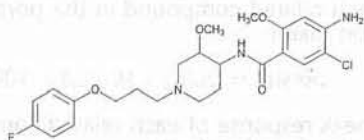


**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Avoid freezing.

• **USP REFERENCE STANDARDS (11)**

- USP Ciprofloxacin Ethylenediamine Analog RS  
1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[(2-aminoethyl)amino]-3-quinolinecarboxylic acid hydrochloride.  
 $C_{15}H_{16}FN_3O_3 \cdot HCl$  341.77  
USP Ciprofloxacin Formamide RS  
1-Cyclopropyl-6-fluoro-7-(4-formyl-1-piperazinyl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid.  
 $C_{18}H_{18}FN_3O_4$  359.35  
USP Ciprofloxacin Hydrochloride RS  
USP Dexamethasone RS  
USP Dexamethasone Acetate RS

**Cisapride**

$C_{23}H_{29}ClFN_3O_4$  465.95  
Benzamide, 4-amino-5-chloro-N-[1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidinyl]-2-methoxy-, *cis*-.  
*cis*-4-Amino-5-chloro-N-[1-[3-(*p*-fluorophenoxy)propyl]-3-methoxy-4-piperidinyl]-*o*-anisamide [81098-60-4].  
Monohydrate 484.0 [260779-88-2].

» Cisapride contains not less than 99.0 percent and not more than 101.0 percent of  $C_{23}H_{29}ClFN_3O_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers, and store at room temperature.

**USP Reference standards (11)**—

USP Cisapride RS  
USP Haloperidol RS

**Completeness of solution (641)**—A solution, 10 mg per mL in methylene chloride, meets the requirements.

**Identification, Infrared Absorption (197K).**

**Specific rotation (781S):** between  $-10^\circ$  and  $+10^\circ$ , measured at  $20^\circ$ .

*Test solution:* 10 mg per mL in methylene chloride.

**Water Determination, Method I (921):** between 3.4% and 4.0%.

**Residue on ignition (281):** not more than 0.1%.

**Chromatographic purity—**

*Solution A*—Prepare a 20 g per L solution of tetrabutylammonium hydrogen sulfate in water.

*Solution B*—Use methanol.

*Mobile phase*—Use variable mixtures of *Solution A* and *Solution B* as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography (621)*).

*Blank solution*—Use methanol.

*System suitability solution*—Prepare a solution of USP Cisapride RS and USP Haloperidol RS in methanol containing about 0.05 mg per mL and 0.4 mg per mL, respectively.

*Test solution 1*—Dissolve an accurately weighed quantity of Cisapride, in methanol to obtain a solution having a known concentration of about 10 mg per mL.

*Test solution 2*—Dilute quantitatively and stepwise *Test solution 1* in methanol to obtain a solution having a known concentration of about 0.05 mg per mL.

*Chromatographic system* (see *Chromatography (621)*)—The liquid chromatograph is equipped with a 275-nm detector and a 4.0-mm  $\times$  10-cm column that contains 3- $\mu$ m base-deactivated packing L1. The flow rate is about 1.2 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–20	80→55	20→45	linear gradient
20–21	55→5	45→95	linear gradient
21–25	5	95	isocratic
25–26	5→80	95→20	return to initial conditions
26–30	80	20	re-equilibration

Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the order of elution is cisapride followed by haloperidol, the resolution,  $R$ , between these two peaks is not less than 2.5; and the relative standard deviation for replicate injections is not more than 2.0% for the cisapride peak.

*Procedure*—Inject a volume (about 10  $\mu$ L) of the *Blank solution*, *Test solution 1*, and *Test solution 2* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of cisapride impurities in the portion of Cisapride taken by the formula:

$$100(C_s / C_i)(r_i / r_s)$$

in which  $C_s$  and  $C_i$  are the concentration of cisapride, in mg per mL, of *Test solution 2* and *Test solution 1*, respectively;  $r_i$  is the individual peak response of cisapride impurities in *Test solution 1*; and  $r_s$  is cisapride peak area in *Test solution 2*: not more than 0.5% of any cisapride impurity is found, and not more than 1.0% of total impurities is found. Disregard any peak also found in the *Blank solution* and any peak with an area less than 0.1 times the area of the principal peak in the *Test solution 2* chromatogram.

**Assay**—Dissolve about 0.350 g of Cisapride, accurately weighed, in 70 mL of a mixture of methyl ethyl ketone and acetic acid (7:1). Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction (see *Titrimetry (541)*). Each mL of 0.1 N perchloric acid is equivalent to 46.60 mg of  $C_{23}H_{29}ClFN_3O_4$ .

**Cisapride Compounded Injection, Veterinary****DEFINITION**

Cisapride Compounded Injection, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of cisapride ( $C_{23}H_{29}ClFN_3O_4$ ), calculated on the anhydrous basis.

Prepare Cisapride Compounded Injection, Veterinary 3 mg/mL as follows (see *Pharmaceutical Compounding—Sterile Preparations (797)*).

Cisapride (as cisapride monohydrate) powder	30 mg (31.2 mg)
Tartaric acid 10% solution	0.75 mL
Sterile Water for Injection, a sufficient amount to make	10 mL

Dissolve the *Cisapride monohydrate powder* in 9 mL of *Sterile Water for Injection*. Add the *Tartaric acid 10% solution*. Add



sufficient *Sterile Water for Injection* to bring to final volume, and mix well. Pass through a sterile filter of 0.22- $\mu$ m pore size into a sterile single-dose container. [NOTE—Tartaric acid is added to aid in solubility.]

## ASSAY

### • PROCEDURE

**Solution A:** Add 0.2 mL of triethylamine to 1000 mL of water.

**Mobile phase:** Acetonitrile and *Solution A* (65:35)

**Standard solution:** 0.2 mg/mL of cisapride prepared from USP Cisapride RS in methanol

**Sample solution:** Transfer 0.67 mL of Injection, Veterinary to a 10-mL volumetric flask, dilute with methanol to volume, and sonicate to mix.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV-Vis 308 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 10  $\mu$ L

### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for cisapride is about 2.0 min.]

### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cisapride ( $C_{23}H_{29}ClFN_3O_4$ ) in the portion of Injection, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cisapride from the *Sample solution*

$r_S$  = peak response of cisapride from the *Standard solution*

$C_S$  = concentration of USP Cisapride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cisapride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0% on the anhydrous basis

## SPECIFIC TESTS

• **PH** (791): 2.1–3.1

• **STERILITY TESTS** (71): It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Membrane Filtration*.

• **BACTERIAL ENDOTOXINS TEST** (85): NMT 5.0 USP Endotoxin Units/mg

• **PARTICULATE MATTER IN INJECTIONS** (788): It meets the requirements.

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Package in single-dose sterile glass containers and store in a refrigerator (2°–8°).

• **LABELING:** Label it to indicate that it is for veterinary use only and to state the *Beyond-Use Date*.

• **BEYOND-USE DATE:** In the absence of performing and completing a sterility and endotoxins test, the storage conditions for *High-Risk Level CSPs in Pharmaceutical Compounding—Sterile Preparations* (797) apply.

After successful completion of sterility and endotoxin testing, NMT 90 days after the date on which it was compounded when stored in a refrigerator (2°–8°).

## • USP REFERENCE STANDARDS (11)

USP Cisapride RS

USP Endotoxin RS

## Cisapride Compounded Oral Suspension, Veterinary

### DEFINITION

Cisapride Compounded Oral Suspension, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of cisapride ( $C_{23}H_{29}ClFN_3O_4$ ), calculated on the anhydrous basis.

Prepare Cisapride Compounded Oral Suspension, Veterinary 10 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Cisapride (as cisapride monohydrate) powder	1 g (1.04 g)
Vehicle: A 1:1 mixture of Ora-Plus <sup>a</sup> and Ora-Sweet <sup>a</sup> , a sufficient quantity to make	100 mL

<sup>a</sup>Perrigo Laboratories, Allegan, MI.

Pour the *Cisapride monohydrate powder* into a suitable container. Wet the powder with a small amount of *Vehicle* and triturate to make a smooth paste. Add the *Vehicle* to make the mortar contents pourable. Transfer contents stepwise and quantitatively to a calibrated container using the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

## ASSAY

### • PROCEDURE

**Solution A:** Add 0.2 mL of triethylamine to 1000 mL of water.

**Mobile phase:** Acetonitrile and *Solution A* (65:35)

**Standard solution:** 0.2 mg/mL of cisapride prepared from USP Cisapride RS in methanol

**Sample solution:** Transfer 1.0 mL of Oral Suspension, Veterinary to a 50-mL volumetric flask, dilute with methanol to volume, and sonicate to mix. Filter into an HPLC vial.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV-Vis 308 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 10  $\mu$ L

### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for cisapride is about 2.0 min.]

### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0% for replicate injections

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cisapride ( $C_{23}H_{29}ClFN_3O_4$ ) in the portion of Oral Suspension, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cisapride from the *Sample solution*

$r_S$  = peak response of cisapride from the *Standard solution*



$C_S$  = concentration of USP Cisapride RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of cisapride in the Sample solution (mg/mL)

Acceptance criteria: 90.0%–110.0% on the anhydrous basis

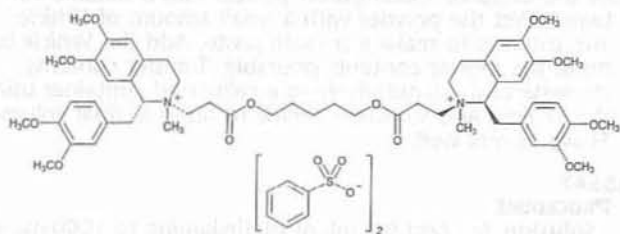
#### SPECIFIC TESTS

- **PH (791):** 4.0–5.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant plastic containers. Store in a refrigerator (2°–8°) or at controlled room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only. Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator (2°–8°) or at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Cisapride RS

### Cisatracurium Besylate



$C_{65}H_{82}N_2O_{18}S_2$  1243.48  
Isoquinolinium, 2,2'-[1,5-pentanediy]bis[oxy(3-oxo-3,1-propanediyl)]bis[1-[(3,4-dimethoxyphenyl)methyl]-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-dibenzesulfonate, [1R-[1 $\alpha$ ,2 $\alpha$ (1'R\*,2'R\*)]]-; (1R,2R)-2-(2-Carboxyethyl)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium benzenesulfonate, pentamethylene ester [96946-42-8].

#### DEFINITION

Cisatracurium Besylate contains NLT 97.0% and NMT 102.0% of cisatracurium besylate ( $C_{65}H_{82}N_2O_{18}S_2$ ), calculated on the anhydrous and solvent-free basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

#### ASSAY

##### PROCEDURE

**Buffer:** 33.3 g/L of ammonium formate prepared as follows. Dissolve 32.8 g of ammonium formate in 984 mL of water, and add 16 mL of anhydrous formic acid.

**Mobile phase:** Acetonitrile, methanol, and Buffer (20:20:60)

**Diluent:** Acetonitrile, methanol, and water (20:20:60). Add 0.4 mL of anhydrous formic acid per 1 L.

**System suitability solution:** 0.7 mg/mL of USP Cisatracurium Besylate System Suitability Mixture RS in Diluent

**Standard solution:** 0.7 mg/mL of USP Cisatracurium Besylate RS in Diluent

**Sample solution:** 0.7 mg/mL of Cisatracurium Besylate in Diluent

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  25.0-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20  $\mu$ L

**Run time:** NLT 2.5 times the retention time of cisatracurium

#### System suitability

**Samples:** System suitability solution and Standard solution

[NOTE—See Table 1 for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between the peaks for *R*-cis-*R'*-trans-atracurium and cisatracurium, System suitability solution

**Tailing factor:** NMT 1.7 for cisatracurium, System suitability solution

**Relative standard deviation:** NMT 1.5%, Standard solution

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of cisatracurium besylate ( $C_{65}H_{82}N_2O_{18}S_2$ ) in the portion of Cisatracurium Besylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Cisatracurium Besylate RS in the Standard solution (mg/mL)

$C_U$  = concentration of Cisatracurium Besylate in the Sample solution (mg/mL)

Acceptance criteria: 97.0%–102.0% on the anhydrous and solvent-free basis

#### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

#### Change to read:

##### ORGANIC IMPURITIES

Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

#### Analysis

**Sample:** Sample solution

Calculate the percentage of each impurity in the portion of Cisatracurium Besylate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the Sample solution

$r_T$  = sum of all the peak responses from the Sample solution

Acceptance criteria: See Table 1. Disregard any peak representing less than 0.09% of the area of the major peak.



Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Benzenesulfonic acid <sup>a</sup> (IRA 1-Jul-2016) <sup>a</sup>	0.10	—
cis-Quaternary acid <sup>b</sup>	0.14	0.2
(R)-N-Methylaudanosine <sup>c</sup>	0.16	0.2
(R)-Laudanosine <sup>d</sup>	0.20	0.6
cis-Quaternary methyl ester <sup>e</sup>	0.23	0.4
cis-Quaternary alcohol <sup>f</sup>	0.29	0.5
R-trans-R'-trans-Atracurium <sup>g</sup>	0.74	0.2
R-cis-R'-trans-Atracurium <sup>h</sup>	0.87	0.8
Cisatracurium	1.0	—
trans-Monoquaternary compound <sup>i</sup>	1.17	0.5
trans-Monoacrylate <sup>j</sup>	1.28	0.5
cis-Monoquaternary compound <sup>k</sup>	1.39	0.7
cis-cis-Triester analog <sup>l</sup>	1.46	0.4
cis-Monoacrylate <sup>m</sup>	1.56	1.0
Any individual unspecified impurity	—	0.1
Total impurities	—	3.0

<sup>a</sup> This peak is due to the counterion and is not to be reported or included in total impurities.

<sup>b</sup> (1R,2R)-2-(2-Carboxyethyl)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium. (IRA 1-Jul-2016)

<sup>c</sup> (R)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2,2-dimethyl-1-veratrylisoquinolinium. (IRA 1-Jul-2016)

<sup>d</sup> (R)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinoline.

<sup>e</sup> (1R,2R)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2-[2-(methoxycarbonyl)ethyl]-2-methyl-1-veratrylisoquinolinium. (IRA 1-Jul-2016)

<sup>f</sup> (1R,2R)-1,2,3,4-Tetrahydro-2-(9-hydroxy-3-oxo-4-oxanonyl)-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium. (IRA 1-Jul-2016)

<sup>g</sup> (1R,1'R,2S,2'S)-2,2'-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium). (IRA 1-Jul-2016)

<sup>h</sup> (1R,1'R,2R,2'S)-2,2'-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium). (IRA 1-Jul-2016)

<sup>i</sup> (1R,1'R,2S)-2-Methyl-2,2'-(3,11-dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-1-veratrylisoquinolinium). (IRA 1-Jul-2016)

<sup>j</sup> (1R,2S)-2-(3,11-Dioxo-4,10-dioxatridecenyloxy)-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium). (IRA 1-Jul-2016)

<sup>k</sup> (1R,1'R,2R)-2-Methyl-2,2'-(3,11-dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-1-veratrylisoquinolinium). (IRA 1-Jul-2016)

<sup>l</sup> (1R,1'R,2R,2'R)-2,2'-(3,7,15-Trioxo-4,8,14-trioxahexadecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium). (IRA 1-Jul-2016)

<sup>m</sup> (1R,2R)-2-(3,11-Dioxo-4,10-dioxatridecenyloxy)-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium). (IRA 1-Jul-2016)

#### • LIMIT OF METHYL BENZENESULFONATE

[NOTE—Prepare the solutions immediately before use. Methyl benzenesulfonate is slowly hydrolyzed in aqueous solutions.]

**Mobile phase:** Acetonitrile and water (45:55)

**Internal standard solution:** 1.2 mg/mL of methyl *p*-toluenesulfonate in acetonitrile

**Standard stock solution A:** 1.2 mg/mL of methyl benzenesulfonate in acetonitrile

**Standard stock solution B:** 48 µg/mL of methyl benzenesulfonate from *Standard stock solution A* in *Mobile phase* prepared as follows. Transfer 2.0 mL of *Standard stock solution A* and 5.0 mL of *Internal standard solution* to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Standard solution:** 2.4 µg/mL of methyl benzenesulfonate from *Standard stock solution B* in *Mobile phase*

**Sample solution:** Nominally 0.2 g/mL of Cisatracurium Besylate in *Mobile phase* prepared as follows. Transfer 1.0 g of Cisatracurium Besylate into a suitable separator.

Immediately add 25 µL of *Internal standard solution*, and dissolve the contents in 25 mL of water using vigorous shaking. Add 25 mL of ethyl acetate, and shake vigorously for 2 min. Allow the phases to separate until the aqueous layer is clear and for NMT 2 h. Evaporate the organic layer to dryness in a current of air. Dissolve the residue in 5.0 mL of *Mobile phase* by sonication and gentle swirling.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 214 nm

**Column:** 4.6-mm × 25.0-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**Run time:** 5 times the retention time of methyl benzenesulfonate

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between methyl benzenesulfonate and methyl *p*-toluenesulfonate

**Relative standard deviation:** NMT 2.0% for methyl benzenesulfonate and methyl *p*-toluenesulfonate

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the concentration of methyl benzenesulfonate in the portion of Cisatracurium Besylate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

$R_U$  = peak height ratio of methyl benzenesulfonate to the internal standard from the *Sample solution*

$R_S$  = peak height ratio of methyl benzenesulfonate to the internal standard from the *Standard solution*

$C_S$  = concentration of methyl benzenesulfonate in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of Cisatracurium Besylate in the *Sample solution* (g/mL)

**Acceptance criteria:** NMT 10 ppm

#### SPECIFIC TESTS

##### • OPTICAL ROTATION (781S), *Procedures, Specific Rotation*

**Sample solution:** 10.0 mg/mL in acetonitrile

**Acceptance criteria:** −60.0° to −54.0° at 20°, calculated on the anhydrous and solvent-free basis

#### Delete the following:

##### • PH (791)

**Sample solution:** 7 mg/mL of Cisatracurium Besylate in water

**Acceptance criteria:** 5.0–6.5 (IRA 1-Jul-2016)

#### Change to read:

##### • WATER DETERMINATION (921) (IRA 1-Jul-2016): NMT 5.0%.

[NOTE—*Method 1a* or *Method 1c* may be used.] (IRA 1-Jul-2016)

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store cold, desiccated.

#### Change to read:

##### • USP REFERENCE STANDARDS (11)

USP Cisatracurium Besylate RS

USP Cisatracurium Besylate System Suitability Mixture RS

Cisatracurium besylate.

*R-trans-R'-trans-Atracurium* •besylate: (IRA 1-Jul-2016)



(1*R*,1'*R*,2*S*,2'*S*)-2,2'-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate.

$C_{65}H_{82}N_2O_{18}S_2$  1243.48

*R*-cis-*R'*-trans-Atracurium besylate: (IRA, 1-Jul-2016)

(1*R*,1'*R*,2*R*,2'*S*)-2,2'-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate.

$C_{65}H_{82}N_2O_{18}S_2$  1243.48

Other related compounds.

## Cisatracurium Besylate Injection

### DEFINITION

Cisatracurium Besylate Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of cisatracurium ( $C_{53}H_{72}N_2O_{12}$ ). Multiple-dose containers may contain benzyl alcohol.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197)

**Standard solution:** 3 mg/mL of USP Cisatracurium Besylate RS in methylene chloride

**Sample solution**

[NOTE—If an emulsion forms during the extraction with methylene chloride, use a centrifuge to separate the layers.]

**For Injections that do not contain benzyl alcohol:**

Nominally 2 mg/mL of cisatracurium from the Injection in methylene chloride prepared as follows. Transfer an amount of Injection equivalent to 10 mg of cisatracurium into a suitable separator, and extract with 5 mL of methylene chloride. Use the lower methylene chloride layer.

**For Injections containing benzyl alcohol:** Nominally 2 mg/mL of cisatracurium from the Injection in methylene chloride prepared as follows. Transfer an amount of Injection equivalent to 10 mg of cisatracurium into a suitable separator, and extract with 20.0 mL of ethyl acetate. Transfer the lower aqueous layer into another separator, and extract with 5 mL of methylene chloride. Use the lower methylene chloride layer.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Add several drops of the *Standard solution* and *Sample solution* to separate potassium bromide plates. Allot the solvent to evaporate, and analyze the resulting residue.

**Acceptance criteria:** Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Solution A:** Acetonitrile, methanol, water, and anhydrous formic acid (21:21:60:1)

**Mobile phase:** 19.4 g/L of ammonium formate in *Solution A*. Filter under vacuum using a suitable filter.

**Diluent:** Acetonitrile, methanol, and water (20:20:60). Add 0.4 mL of anhydrous formic acid per 1 L.

**System suitability solution:** 0.7 mg/mL of USP Cisatracurium Besylate System Suitability Mixture RS in *Diluent*

**Standard solution:** 0.7 mg/mL of USP Cisatracurium Besylate RS in *Diluent*

**Sample solution:** Nominally 0.7 mg/mL of cisatracurium besylate from the Injection in *Diluent*, equivalent

to 0.5 mg/mL of cisatracurium, prepared as follows. Using a "to contain" pipet, transfer a suitable volume of Injection to an appropriate volumetric flask, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25.0-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between *R*-cis-*R'*-trans-atracurium and cisatracurium, *System suitability solution*

**Tailing factor:** NMT 1.7 for cisatracurium, *Standard solution*

**Relative standard deviation:** NMT 1.5%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cisatracurium ( $C_{53}H_{72}N_2O_{12}$ ) in the portion of Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (M_{r1}/M_{r2}) \times 100$$

$r_u$  = peak response of cisatracurium from the *Sample solution*

$r_s$  = peak response of cisatracurium from the *Standard solution*

$C_s$  = concentration of USP Cisatracurium Besylate RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of cisatracurium in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of cisatracurium, 929.14

$M_{r2}$  = molecular weight of cisatracurium besylate, 1243.48

**Acceptance criteria:** 90.0%–110.0%

### OTHER COMPONENTS

#### • BENZYL ALCOHOL CONTENT (IF PRESENT)

**Internal standard solution:** 4.5 mg/mL of decanol in ethyl acetate

**Standard stock solution:** 9.0 mg/mL of USP Benzyl Alcohol RS in water

**Standard solution:** 0.45 mg/mL of benzyl alcohol from the *Standard stock solution* prepared as follows. Transfer 5.0 mL of *Standard stock solution* and 20.0 mL of ethyl acetate into a 50-mL centrifuge tube. Stopper, shake well for 30 s, and allow the phases to separate. Transfer 10.0 mL of the top (organic) layer and 5.0 mL of the *Internal standard solution* into a 50-mL volumetric flask. Dilute with ethyl acetate to volume.

**Sample solution:** Transfer 5.0 mL of the Injection and 20.0 mL of ethyl acetate into a 50-mL centrifuge tube. Stopper, shake well for 30 s, and allow the phases to separate. Transfer 10.0 mL of the top (organic) layer and 5.0 mL of the *Internal standard solution* into a 50-mL volumetric flask. Dilute with ethyl acetate to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm × 15-m fused-silica capillary column coated with a 1.0-μm layer of liquid phase G16



**Temperatures**

Detector: 250°

Injection port: 220°

Column: 140°

Carrier gas: Helium

Flow rate: 5.0 mL/min

Injection volume: 1 µL

Injection type: Split ratio, 2.5: 1

**System suitability**Sample: *Standard solution*

[NOTE—The relative retention times for decanol and benzyl alcohol are 0.62 and 1.0, respectively.]

**Suitability requirements**

Resolution: NLT 2.0 between decanol and benzyl alcohol

Relative standard deviation: NMT 2.0% for peak area ratios of benzyl alcohol to decanol

**Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzyl alcohol in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/L) \times 100$$

$R_U$  = peak area ratio of benzyl alcohol to the internal standard from the *Sample solution*

$R_S$  = peak area ratio of benzyl alcohol to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Benzyl Alcohol RS in the *Standard stock solution* (mg/mL)

$L$  = label claim of benzyl alcohol (mg/mL)

Acceptance criteria: 90.0%–110.0% of the labeled amount of benzyl alcohol

**IMPURITIES****• ORGANIC IMPURITIES**

Mobile phase, Diluent, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

Sensitivity solution: 0.4 µg/mL of USP Cisatracurium Besylate RS in Diluent

**Analysis**

Samples: *Standard solution*, *Sample solution*, and *Sensitivity solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response for each impurity from the *Sample solution*

$r_S$  = peak response of cisatracurium from the *Standard solution*

$C_S$  = concentration of USP Cisatracurium Besylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cisatracurium in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of cisatracurium, 929.14

$M_{r2}$  = molecular weight of cisatracurium besylate, 1243.48

Acceptance criteria: See Table 1. Disregard any peaks with responses less than the peak from the *Sensitivity solution*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Besylate <sup>a</sup>	0.10	—
cis-Quaternary acid <sup>b</sup>	0.13	4.3
(R)-N-Methylaudanosine <sup>c,d</sup>	0.15	—
(R)-Laudanosine <sup>e</sup>	0.19	4.0
cis-Quaternary methyl ester <sup>f,d</sup> and benzyl alcohol <sup>g</sup>	0.22	—
cis-Quaternary alcohol <sup>h</sup>	0.27	5.0
Benzaldehyde <sup>i</sup>	0.40	—
R-trans-R'-trans-Atracurium <sup>j,d</sup>	0.72	—
R-cis-R'-trans-Atracurium <sup>k,d</sup>	0.88	—
Cisatracurium	1.0	—
trans-Monoquaternary compound <sup>l,d</sup>	1.19	—
trans-Monoacrylate <sup>m,d</sup>	1.30	—
cis-Monoquaternary compound <sup>n,d</sup>	1.42	—
cis-cis-Triester analog <sup>o,d</sup>	1.47	—
cis-Monoacrylate <sup>p</sup>	1.58	2.5
Any individual unspecified degradation product	—	0.2
Total degradation products	—	14.4

<sup>a</sup> This peak is due to the counterion and is not to be reported or included in *Total degradation products*.

<sup>b</sup> (1R,2R)-2-(2-Carboxyethyl)-1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisquinolinium benzenesulfonate.

<sup>c</sup> (R)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2,2-dimethyl-1-veratrylisquinolinium benzenesulfonate.

<sup>d</sup> This is a process impurity that is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and is not to be included in the total degradation products.

<sup>e</sup> (R)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisquinoline.

<sup>f</sup> (1R,2R)-1,2,3,4-Tetrahydro-6,7-dimethoxy-2-[2-(methoxycarbonyl)ethyl]-2-methyl-1-veratrylisquinolinium benzenesulfonate.

<sup>g</sup> Benzyl alcohol co-elutes with cis-quaternary methyl ester. It is monitored using the test for Benzyl Alcohol Content.

<sup>h</sup> (1R,2R)-1,2,3,4-Tetrahydro-2-(9-hydroxy-3-oxo-4-oxanonyl)-6,7-dimethoxy-2-methyl-1-veratrylisquinolinium benzenesulfonate.

<sup>i</sup> Benzaldehyde is a degradant of benzyl alcohol and is not included in the total impurities.

<sup>j</sup> (1R,1'R,2S,2'S)-2,2'-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisquinolinium) dibenzenesulfonate.

<sup>k</sup> (1R,1'R,2R,2'S)-2,2'-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisquinolinium) dibenzenesulfonate.

<sup>l</sup> (1R,1'R,2S)-2-Methyl-2,2'-(3,11-dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-1-veratrylisquinolinium) dibenzenesulfonate.

<sup>m</sup> (1R,2S)-2-(3,11-Dioxo-4,10-dioxo-12-tridecenyl)-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisquinolinium) benzenesulfonate.

<sup>n</sup> (1R,1'R,2R)-2-Methyl-2,2'-(3,11-dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-1-veratrylisquinolinium) dibenzenesulfonate.

<sup>o</sup> (1R,1'R,2R,2'R)-2,2'-(3,7,15-Trioxo-4,8,14-trioxahexadecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisquinolinium) dibenzenesulfonate.

<sup>p</sup> (1R,2R)-2-(3,11-Dioxo-4,10-dioxo-12-tridecenyl)-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisquinolinium) benzenesulfonate.

**SPECIFIC TESTS**

• **PH (791):** 3.0–3.8

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 8.17 USP Endotoxin Units/mg of cisatracurium besylate

• **STERILITY TESTS (71):** Meets the requirements for the test for *Sterility of the Product to Be Examined, Membrane Filtration*

• **OTHER REQUIREMENTS:** Meets the requirements for *Injections and Implanted Drug Products (1)*



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store in a cool place, protected from freezing and light.

- **USP REFERENCE STANDARDS (11)**

USP Benzyl Alcohol RS  
 USP Cisatracurium Besylate RS  
 USP Cisatracurium Besylate System Suitability Mixture RS  
 Cisatracurium besylate.  
*R-trans-R'-trans-Atracurium:*  
 1*R*,1'*R*,2*S*,2'*S*)-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate.  
 $C_{65}H_{82}N_{20}O_{18}S_2$  1243.48  
*R-cis-R'-trans-Atracurium:*  
 (1*R*,1'*R*,2*R*,2'*S*)-(3,11-Dioxo-4,10-dioxatridecamethylene)bis(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methyl-1-veratrylisoquinolinium) dibenzenesulfonate.  
 $C_{65}H_{82}N_{20}O_{18}S_2$  1243.48  
 Other related compounds.  
 USP Endotoxin RS

Application volume: 5  $\mu$ L

Developing solvent system: Acetone and 1 N nitric acid (9:1)

Spray reagent A: Mix *Solution A* and *Solution B* together. Disregard any precipitate that is formed. Store in the dark.

[NOTE—The solution is usable for at least 1 week.]

Spray reagent B: 20 mg/mL of potassium iodide in water

**Analysis**

Samples: *Standard solution* and *Sample solution*

Develop with *Developing solvent system*, in a suitable chromatographic chamber containing a filter paper lining and equilibrated for 30 min with the *Developing solvent system*, for a distance of about 8 cm from the origin, followed by air drying. Complete the drying by heating in a forced-air oven at about 100° for 1 min. Spray with *Spray reagent A*, heat in an oven at about 100° for 5 min, cool, and spray with *Spray reagent B*, to bring out the full color of the spots.

Acceptance criteria: The principal spot from the *Sample solution* corresponds in appearance and  $R_f$  value to that produced by the *Standard solution*.  $\Delta$ USP40

**ASSAY****Change to read:**

- **PROCEDURE**

Mobile phase: Ethyl acetate, methanol, dimethylformamide, and degassed water (25:16:5:5)

Standard solution: 1 mg/mL of USP Cisplatin RS in dimethylformamide. Use within 1 h.

Sample solution: 1 mg/mL of Cisplatin in dimethylformamide.  $\Delta$ Use within 1 h.  $\Delta$ USP40

Chromatographic system  
 (See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 310 nm

Column: 4.0-mm  $\times$  30-cm; packing L8

Flow rate: 2.0 mL/min

Injection volume: 40  $\mu$ L

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT  $\Delta$ 0.73%  $\Delta$ USP40

**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cisplatin ( $Cl_2H_6N_2Pt$ ) in the portion of Cisplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cisplatin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cisplatin in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

**IMPURITIES****Change to read:**

- **LIMIT OF TRICHLOROAMMINEPLATINATE**

Mobile phase: 0.4 g/L of ammonium sulfate in water.  $\Delta$ Adjust with 6 N ammonium hydroxide to a pH of 5.9.

$\Delta$ USP40

Standard solution: 6  $\mu$ g/mL of USP Potassium Trichloroammineplatinate RS in saline TS.  $\Delta$ Protect the solution from light.  $\Delta$ USP40 Use the solution within 4 h.

**Cisplatin**

$Cl_2H_6N_2Pt$  300.05  
 Platinum, diamminedichloro-, (SP-4-2);  
*cis*-Diamminedichloroplatinum [15663-27-1].

**DEFINITION**

Cisplatin contains NLT 98.0% and NMT 102.0% of cisplatin ( $Cl_2H_6N_2Pt$ ), calculated on the anhydrous basis.

[CAUTION—Cisplatin is potentially cytotoxic. Great care should be taken to prevent inhaling particles and exposing the skin to it.]

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**Change to read:**

- **B.  $\Delta$ INFRARED ABSORPTION (197)**

[NOTE—Methods described in (197K) or (197A) may be used.]  $\Delta$ USP40

**Delete the following:**

- **C. THIN-LAYER CHROMATOGRAPHY**

Standard solution: 1 mg/mL of USP Cisplatin RS in dimethylformamide

Sample solution: 1 mg/mL of Cisplatin in dimethylformamide

Solution A: 5.6 g of stannous chloride in 10 mL of hydrochloric acid. Stir for 5 min. [NOTE—It is not necessary that all of the solids dissolve.]

Solution B: 0.2 g of potassium iodide in 90 mL of water

Chromatographic system  
 (See *Chromatography* (621), *Thin-Layer Chromatography*.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture



**Sample solution:** 0.5 mg/mL of Cisplatin in saline TS. Completely dissolve by stirring by mechanical means for 30 min. <sup>▲</sup>Protect the solution from light. <sup>▲</sup>USP40 Use the solution within 4 h.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 209 nm

**Column:** 4.6-mm × 25-cm; packing L14

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—<sup>▲</sup>The relative retention times for saline and trichloroamineplatinate are about 0.4 and 1.0, respectively. <sup>▲</sup>USP40]

#### Suitability requirements

**Resolution:** NLT 2.0 between the saline and trichloroamineplatinate peaks

**Relative standard deviation:** NMT 3.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of trichloroamineplatinate in the portion of Cisplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak area of trichloroamineplatinate from the *Sample solution*

$r_S$  = peak area of trichloroamineplatinate from the *Standard solution*

$C_S$  = concentration of USP Potassium Trichloroamineplatinate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cisplatin in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of trichloroamineplatinate, 318.48

$M_{r2}$  = molecular weight of potassium trichloroamineplatinate, 357.58

**Acceptance criteria:** NMT 1.0%

#### Change to read:

#### • LIMIT OF TRANSPLATIN

**Mobile phase:** 0.18 M monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.2.

**Standard stock solution A:** 0.05 mg/mL of USP Transplatin RS in saline TS. Dissolve by stirring by mechanical means for 30 min.

**Standard stock solution B:** Transfer 5 mL of *Standard stock solution A* to a 25-mL volumetric flask containing 12 mg of USP Cisplatin RS. Dilute with saline TS to volume, and stir by mechanical means for 30 min to dissolve.

**System suitability stock solution:** 0.05 mg/mL of USP Cisplatin RS in saline TS. Dissolve by stirring by mechanical means for 30 min.

**System suitability solution:** Transfer 10 mL each of *System suitability stock solution* and *Standard stock solution A* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytetrafluoroethylene-lined closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

**Standard solution:** Transfer 10 mL of *Standard stock solution B* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytetrafluoroethylene-lined closure,

and heat in a heating block at 60 ± 0.5° for 60 min.

Remove, and cool to room temperature.

**Sample stock solution:** 0.5 mg/mL of Cisplatin in saline TS. Dissolve by stirring by mechanical means for 30 min.

**Sample solution:** Transfer 10 mL of *Sample stock solution* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytetrafluoroethylene-lined closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L9

**Column temperature:** 45°

**Flow rate:** 2.0 mL/min

**Injection volume:** 20 µL

[NOTE—Condition the *Column* by pumping *Mobile phase* at a flow rate of 2.0 mL/min for 30 min, then at 0.5 mL/min for 30 min, and then again at 2.0 mL/min for 30 min.]

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The retention time for derivatized transplatin is between 5.0 and 9.0 min; or, if it is not, modify the *Mobile phase* as necessary, and recondition the *Column*. The relative retention times for <sup>▲</sup>derivatized <sup>▲</sup>USP40 cisplatin and <sup>▲</sup>derivatized <sup>▲</sup>USP40 transplatin are about 1.0 and 1.3, respectively.]

#### Suitability requirements

<sup>▲</sup>USP40

**Resolution:** NLT 1.7 <sup>▲</sup>between the derivatized cisplatin and derivatized transplatin peaks, <sup>▲</sup>USP40 *System suitability solution*

**Relative standard deviation:** NMT 4.0% <sup>▲</sup>for the derivatized transplatin peak, <sup>▲</sup>USP40 *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of transplatin in the portion of Cisplatin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of <sup>▲</sup>derivatized <sup>▲</sup>USP40 transplatin from the *Sample solution*

$r_S$  = peak area of <sup>▲</sup>derivatized <sup>▲</sup>USP40 transplatin from the *Standard solution*

$C_S$  = concentration of USP Transplatin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cisplatin in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 2.0%

#### • UV PURITY RATIO

Cleanse all glassware with a mixture of hydrochloric acid and nitric acid (3:1), rinse thoroughly with water, and dry before use. Do not use dichromate for cleaning. Do not use acetone or pressurized air for drying.

**Sample:** 98.5 ± 0.5 mg of ground Cisplatin

#### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV

**Analytical wavelength:** Maxima near 301 nm and minima near 246 nm

**Cell:** 2 cm

**Blank:** 0.1 N hydrochloric acid

**Analysis:** Transfer the *Sample* to a 100-mL volumetric flask, and add 0.1 N hydrochloric acid to volume. Using a clean magnetic stir bar, alternately stir at a high speed for 5 min and sonicate for 10 s until complete solution is effected, inverting the flask frequently to re-



move particles that may cling to the neck. Protect this solution from light, and use within 1 h of preparation. Obtain the absorption spectrum. Calculate the absorbance ratio as follows:

$$\text{Result} = A_{301}/A_{246}$$

$A_{301}$  = absorbance at near 301 nm

$A_{246}$  = absorbance at near 246 nm

Acceptance criteria: NLT 4.5

## SPECIFIC TESTS

### Change to read:

#### • PLATINUM CONTENT

▲Sample: 0.5 g of Cisplatin

Analysis: Ignite the *Sample* to constant weight at  $800 \pm 50^\circ$ , and weigh the residue. The residue is platinum.

Calculate the platinum content in the portion of Cisplatin taken:

$$\text{Result} = (W_U/W_S) \times 100$$

$W_U$  = weight of platinum

$W_S$  = weight of the *Sample*

Acceptance criteria: 64.42%–65.22% on the anhydrous basis.▲USP40

• CRYSTALLINITY (695): Meets the requirements

• WATER DETERMINATION (921), Method I: NMT 1.0%

## ADDITIONAL REQUIREMENTS

### Change to read:

• PACKAGING AND STORAGE: Preserve in tight containers.

Protect from light. ▲Store at room temperature.▲USP40

• USP REFERENCE STANDARDS (11)

USP Cisplatin RS

USP Potassium Trichloroammineplatinate RS

$\text{Cl}_2\text{H}_3\text{KNPt}$  357.58

USP Transplatin RS

## Cisplatin for Injection

### DEFINITION

Cisplatin for Injection is a sterile, lyophilized mixture of Cisplatin, Mannitol, and Sodium Chloride. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cisplatin ( $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$ ).

[CAUTION—Cisplatin is potentially cytotoxic. Great care should be taken in handling the powder and preparing solutions.]

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHY

Solution A: 5.6 g of stannous chloride in 10 mL of hydrochloric acid. Stir for 5 min. [NOTE—It is not necessary that all of the solids dissolve.]

Solution B: 0.2 g of potassium iodide in 90 mL of water  
Standard solution: 1.0 mg/mL of USP Cisplatin RS, 9 mg/mL of sodium chloride, and 10 mg/mL of D-mannitol in water

Sample solution: Nominally equivalent to 1.0 mg/mL of cisplatin by dissolving the contents of 1 container of Cisplatin for Injection in water, based on the label claim

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Mode: TLC

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 5  $\mu\text{L}$

Developing solvent system: Acetone and 1 N nitric acid (9:1)

Spray reagent A: Mix *Solution A* and *Solution B* together. Disregard any precipitate that is formed. Store in the dark. [NOTE—The solution is usable for at least 1 week.]

Spray reagent B: 20 mg/mL of potassium iodide in water

### Analysis

Samples: *Standard solution* and *Sample solution*

Develop with *Developing solvent system*, in a suitable chromatographic chamber containing a filter paper lining and equilibrated for 30 min with the *Developing solvent system*, for a distance of about 8 cm from the origin, followed by air drying. Complete the drying by heating in a forced-air oven at about  $100^\circ$  for 1 min. Spray with *Spray reagent A*, heat in an oven at about  $100^\circ$  for 5 min, cool, and spray with *Spray reagent B* to bring out the full color of the spots.

Acceptance criteria: The principal spot from the *Sample solution* corresponds in appearance and  $R_f$  value to that produced by the *Standard solution*.

### Add the following:

▲• B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.▲USP40

## ASSAY

### Change to read:

#### • PROCEDURE

Mobile phase: Ethyl acetate, methanol, dimethylformamide, and degassed water (25:16:5:5)

Standard solution: 1 mg/mL of USP Cisplatin RS in dimethylformamide. Use within 1 h.

Sample solution: Nominally equivalent to 1.0 mg/mL of cisplatin in dimethylformamide prepared as follows. Quantitatively dissolve 1 container of Cisplatin for Injection with dimethylformamide by sonicating for 5 min. Pass 5 mL of this solution through a suitable filter and collect the filtrate after discarding the first mL of the filtrate.▲Use within 1 h.▲USP40

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 310 nm

Column: 4.0-mm  $\times$  30-cm; packing L8

Flow rate: 2.0 mL/min

Injection volume: 40  $\mu\text{L}$

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cisplatin ( $\text{Cl}_2\text{H}_6\text{N}_2\text{Pt}$ ) in the portion of Cisplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cisplatin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cisplatin in the *Sample solution* (mg/mL)



Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

## IMPURITIES

### Change to read:

- **LIMIT OF TRICHLOROAMMINEPLATINATE**

**Mobile phase:** 0.4 g/L of ammonium sulfate in water. <sup>▲</sup>Adjust with 6 N ammonium hydroxide to a pH of 5.9.

<sup>▲</sup>USP40

**Standard solution:** 6 µg/mL of USP Potassium Trichloroamineplatinate RS in saline TS. <sup>▲</sup>Protect the solution from light. <sup>▲</sup>USP40 Use the solution within 4 h.

**Sample solution:** Nominally equivalent to 0.5 mg/mL of cisplatin by quantitatively dissolving the contents of 1 container of Cisplatin for Injection with water. <sup>▲</sup>Protect the solution from light. Use the solution within 4 h.

<sup>▲</sup>USP40

### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 209 nm

**Column:** 4.6-mm × 25-cm; packing L14

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

### System suitability

**Sample:** *Standard solution*

[NOTE—<sup>▲</sup>The relative retention times for saline and trichloroamineplatinate are about 0.4 and 1.0, respectively. <sup>▲</sup>USP40]

### Suitability requirements

**Resolution:** NLT 2.0 between the saline and trichloroamineplatinate peaks

**Relative standard deviation:** NMT 3.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of trichloroamineplatinate in the portion of Cisplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak area of trichloroamineplatinate from the *Sample solution*

$r_S$  = peak area of trichloroamineplatinate from the *Standard solution*

$C_S$  = concentration of USP Potassium Trichloroamineplatinate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cisplatin in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of trichloroamineplatinate, 318.48

$M_{r2}$  = molecular weight of potassium trichloroamineplatinate, 357.58

**Acceptance criteria:** NMT 1.0%

### Change to read:

- **LIMIT OF TRANSPLATIN**

**Mobile phase:** 0.18 M monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 3.2.

**Standard stock solution A:** 0.05 mg/mL of USP Transplatin RS in saline TS. Dissolve by stirring by mechanical means for 30 min.

**Standard stock solution B:** Transfer 5 mL of *Standard stock solution A* to a 25-mL volumetric flask containing 12 mg of USP Cisplatin RS. Dilute with saline TS to volume, and stir by mechanical means for 30 min to dissolve.

**System suitability stock solution:** 0.05 mg/mL of USP Cisplatin RS in saline TS. Dissolve by stirring by mechanical means for 30 min.

**System suitability solution:** Transfer 10 mL each of *System suitability stock solution* and *Standard stock solution A* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytetrafluoroethylene closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

**Standard solution:** Transfer 10 mL of *Standard stock solution B* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytetrafluoroethylene closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

**Sample stock solution:** Nominally equivalent to 0.5 mg/mL of cisplatin by dissolving the contents of 1 container of Cisplatin for Injection with water

**Sample solution:** Transfer 10 mL of *Sample stock solution* to a 50-mL volumetric flask. Add 5.0 mL of a solution (5 mg/mL) of thiourea (prepared fresh daily) and 5.0 mL of 1 N hydrochloric acid, and dilute with saline TS to volume. Place 10 mL of this solution in a suitable serum vial, seal with a polytetrafluoroethylene closure, and heat in a heating block at 60 ± 0.5° for 60 min. Remove, and cool to room temperature.

### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6 mm × 25-cm; packing L9

**Column temperature:** 45°

**Flow rate:** 2.0 mL/min

**Injection volume:** 20 µL

[NOTE—Condition the *Column* by pumping the *Mobile phase* at a flow rate of 2.0 mL/min for 30 min, then at 0.5 mL/min for 30 min, and then again at 2.0 mL/min for 30 min.]

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The retention time for derivatized transplatin is between 5.0 and 9.0 min; or, if it is not, modify the *Mobile phase* as necessary, and recondition the *Column*. The relative retention times for <sup>▲</sup>derivatized <sup>▲</sup>USP40 cisplatin and <sup>▲</sup>derivatized <sup>▲</sup>USP40 transplatin are about 1.0 and 1.3, respectively.]

### Suitability requirements

<sup>▲</sup>USP40

**Resolution:** NLT 1.7 <sup>▲</sup>between the derivatized cisplatin and derivatized transplatin peaks, <sup>▲</sup>USP40 *System suitability solution*

**Relative standard deviation:** NMT 4.0% <sup>▲</sup>for the derivatized transplatin peak, <sup>▲</sup>USP40 *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of transplatin in the portion of Cisplatin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of <sup>▲</sup>derivatized <sup>▲</sup>USP40 transplatin from the *Sample solution*

$r_S$  = peak area of <sup>▲</sup>derivatized <sup>▲</sup>USP40 transplatin from the *Standard solution*

$C_S$  = concentration of USP Transplatin RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cisplatin in the *Sample solution* (mg/mL)



Acceptance criteria: NMT 2.0%

## SPECIFIC TESTS

### • PH (791)

Sample: Constituted as directed in the labeling, using Sterile Water for Injection

Acceptance criteria: 3.5–6.2

- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 2.0 USP Endotoxin Units/mg of cisplatin.
- **STERILITY TESTS (71), Test for Sterility of the Product to Be Examined, Membrane Filtration:** Meets the requirements
- **WATER DETERMINATION (921), Method I**

Sample: One container of Cisplatin for Injection

Analysis: Use anhydrous formamide as the extraction solvent, and use the following procedure. Introduce 50 mL of anhydrous formamide into the titration vessel, and titrate with the Reagent to the electrometric endpoint. Use the formamide thus dried to rinse a suitable glass syringe equipped with a 22-gauge needle, about 8 cm long. Add the rinse back to the titration vessel, and again titrate the vessel contents, if necessary. Via the syringe, withdraw 5 mL of the formamide thus titrated, and, through the closure of the container, expel the contents into the container. Shake the container to obtain a solution. With the same syringe, withdraw all of the contents of the container, and transfer to the titration vessel. Titrate to the endpoint, adjusting the feeding speed control to the lowest setting, to avoid over-titration.

Acceptance criteria: NMT 2.0%

- **CONSTITUTED SOLUTIONS:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products (1), Specific Tests, Completeness and clarity of solutions.*
- **OTHER REQUIREMENTS:** It meets the requirements for *Labeling (7), Labels and Labeling for Injectable Products.*

## ADDITIONAL REQUIREMENTS

### Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements (659), Injection Packaging, Packaging for constitution.* (CN 1-May-2017) Protect from light.
- **USP REFERENCE STANDARDS (11)**
  - USP Cisplatin RS
  - USP Endotoxin RS
  - USP Potassium Trichloroammineplatinate RS
  - Cl<sub>3</sub>H<sub>3</sub>KNPt 357.58
  - USP Transplatin RS

## Citalopram Oral Solution

### DEFINITION

Citalopram Oral Solution contains an amount of citalopram hydrobromide equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of citalopram free base (C<sub>20</sub>H<sub>21</sub>FN<sub>2</sub>O). It may contain a suitable preservative.

### IDENTIFICATION

- **A.** The retention time of the citalopram peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

Solution A: Methanol and acetonitrile (1:9)

Buffer: 6.1 g/L of monobasic potassium phosphate in water. Add 1.5 mL of triethylamine per L of the solution. Adjust with phosphoric acid to a pH of 2.5.

Mobile phase: Solution A and Buffer (7:18)

Diluent: Acetonitrile and Buffer (1:3)

Standard solution: 0.25 mg/mL of USP Citalopram Hydrobromide RS

Sample solution: Transfer a suitable volume of Oral Solution to a suitable volumetric flask to obtain 0.2 mg/mL final concentration of citalopram free base. Add 50% of the flask volume of Diluent, and sonicate at room temperature for 3 min with intermittent shaking. Allow the solution to cool, and dilute with Diluent to volume. [NOTE—The Sample solution may be filtered through either a PVDF or nylon membrane filter of suitable pore size.]

### Chromatographic system

(See *Chromatography (621), System Suitability.*)

Mode: LC

Detector: UV 240 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 μL

Run time: 2 times the retention time of citalopram

### System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of citalopram (C<sub>20</sub>H<sub>21</sub>FN<sub>2</sub>O) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of citalopram from the Sample solution

$r_S$  = peak response of citalopram from the Standard solution

$C_S$  = concentration of USP Citalopram Hydrobromide RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of citalopram in the Sample solution (mg/mL)

$M_{r1}$  = molecular weight of citalopram free base, 324.39

$M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

Acceptance criteria: 90.0%–110.0% of citalopram free base (C<sub>20</sub>H<sub>21</sub>FN<sub>2</sub>O)

## IMPURITIES

### • ORGANIC IMPURITIES

Solution A: Acetonitrile, methanol, and tetrahydrofuran (17:1:2)

Buffer: Dissolve 3.0 g of 1-octane sulfonic acid sodium salt in 1 L of water. Add 2 mL of triethylamine and 5 mL of tetra-*n*-butyl ammonium hydroxide, 40 percent in water. Mix, and adjust with phosphoric acid to a pH of 3.0.

Mobile phase: Solution A and Buffer (1:3)

Diluent: Acetonitrile and water (1:3)

System suitability solution: 6 μg/mL of USP

Citalopram Related Compound D RS and 1.3 mg/mL of USP Citalopram Hydrobromide RS in Diluent

Standard solution: 6.3 μg/mL of USP Citalopram Hydrobromide RS in Diluent

Sample solution: Transfer a suitable volume of the Oral Solution to a suitable volumetric flask to obtain 1 mg/mL final concentration of citalopram. Add 60% of the flask volume of Diluent, and sonicate at room temperature for 3 min with intermittent shaking. Allow the solution to cool, and dilute with Diluent to volume. [NOTE—The Sample solution may be filtered through either a PVDF or nylon membrane filter of suitable pore size.]



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 225 nm**Column:** 4.6-mm × 15-cm; 5-μm packing L1**Flow rate:** 1.5 mL**Injection volume:** 20 μL**Run time:** 2.6 times the retention time of citalopram for the *System suitability solution* and *Standard solution*; 5.7 times the retention time of citalopram for the *Sample solution***System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 1.8 between citalopram and citalopram related compound D, *System suitability solution***Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 5.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak response of each individual impurity from the *Sample solution* $r_S$  = peak response of citalopram from the *Standard solution* $C_S$  = concentration of USP Citalopram Hydrobromide RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of citalopram in the *Sample solution* (mg/mL) $F$  = relative response factor for each impurity (see *Table 1*) $M_{r1}$  = molecular weight of citalopram free base, 324.39 $M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30**Acceptance criteria:** See *Table 1*.**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound A <sup>a</sup>	0.29	0.86	0.20
Carboxy citalopram <sup>b</sup>	0.47	0.72	0.20
Desfluorocitalopram <sup>c,d</sup>	0.71	—	—
Citalopram related compound C <sup>e</sup>	0.83	1.8	0.20
Citalopram	1.0	—	—

<sup>a</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.<sup>b</sup> 1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxylic acid.<sup>c</sup> 1-[3-(Dimethylamino)propyl]-1-phenyl-1,3-dihydroisobenzofuran-5-carbonitrile.<sup>d</sup> This process impurity is included in the table for identification only. It is controlled in the drug substance, and is not to be reported or included in the total impurities for the drug product.<sup>e</sup> 3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3H)-isobenzofuranone.<sup>f</sup> 3-[5-Chloro-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-N,N-dimethylpropan-1-amine.<sup>g</sup> 3-[5-Bromo-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-N,N-dimethylpropan-1-amine.**Table 1 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound D	1.11	0.95	0.20
Citalopram related compound G <sup>f,d</sup>	3.13	—	—
Citalopram related compound H <sup>g,d</sup>	3.75	—	—
Any individual, unspecified degradation product	—	1.0	0.15
Total impurities	—	—	0.50

<sup>a</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.<sup>b</sup> 1-[3-(Dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxylic acid.<sup>c</sup> 1-[3-(Dimethylamino)propyl]-1-phenyl-1,3-dihydroisobenzofuran-5-carbonitrile.<sup>d</sup> This process impurity is included in the table for identification only. It is controlled in the drug substance, and is not to be reported or included in the total impurities for the drug product.<sup>e</sup> 3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3H)-isobenzofuranone.<sup>f</sup> 3-[5-Chloro-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-N,N-dimethylpropan-1-amine.<sup>g</sup> 3-[5-Bromo-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-N,N-dimethylpropan-1-amine.**SPECIFIC TESTS****• DELIVERABLE VOLUME (698):** Meets the requirements**• pH (791):** 3.5–7.0**• MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed  $10 \times 10^1$  cfu/mL. The total yeasts and molds count does not exceed  $5 \times 10^1$  cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in light-resistant containers at controlled room temperature.**• USP REFERENCE STANDARDS (11)**

USP Citalopram Hydrobromide RS

USP Citalopram Related Compound D RS

[NOTE—May be available as a hydrochloride or hydrobromide salt.]

1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrochloride.

C<sub>19</sub>H<sub>19</sub>FN<sub>2</sub>O · HCl 346.83

1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrobromide.

C<sub>19</sub>H<sub>19</sub>FN<sub>2</sub>O · HBr 391.28**Citalopram Tablets****DEFINITION**Citalopram Tablets contain an amount of citalopram hydrobromide equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of citalopram free base (C<sub>20</sub>H<sub>21</sub>FN<sub>2</sub>O).**IDENTIFICATION****• A. INFRARED ABSORPTION (197K)****Sample:** Extract finely ground Tablet powder containing 200 mg of citalopram with 30 mL of water, and filter. Add 1 mL of 1 N sodium hydroxide to the filtrate, and extract with 50 mL of cyclohexane by shaking for 10 min. Pass the cyclohexane layer through a silicone-treated filter paper into a beaker. Reduce the filtrate



down to 3 mL, using gentle heat as necessary. Transfer the hot solution to a small centrifuge tube. Induce crystallization while cooling by scratching the side of the test tube with a spatula. Centrifuge the mixture, and decant off the cyclohexane. Dry the residue under vacuum in a desiccator. [NOTE—If crystallization fails to occur in the above procedure, use the following alternative procedure. Extract finely ground Tablet powder containing about 50 mg of citalopram with 10 mL of chloroform in a test tube, and sonicate for 1 min. Centrifuge for 10 min, and filter into a beaker. Evaporate to dryness with nitrogen and, if necessary, induce crystallization by etching the beaker.]

Mix approximately 2 mg of the residue with approximately 300 mg of potassium bromide, and record the IR spectrum.

Acceptance criteria: Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### • PROCEDURE

**Buffer:** 1.42 g/L of anhydrous dibasic sodium phosphate in water

**Diluent:** Methanol and *Buffer* (80:20)

**Mobile phase:** 0.77 mg/mL of dodecyltrimethylammonium bromide in *Diluent*

**Internal standard solution:** 0.25 mg/mL of USP

Citalopram Related Compound F RS in *Diluent*

**Standard stock solution:** 1.25 mg/mL of USP

Citalopram Hydrobromide RS in *Diluent*

**Standard solution:** 0.025 mg/mL of USP Citalopram Related Compound F RS and 0.125 mg/mL of USP

Citalopram Hydrobromide RS from the *Internal standard solution* and the *Standard stock solution*, respectively, in *Diluent*

**Sample solution:** Transfer 10 Tablets to a 200-mL volumetric flask, add 25 mL of *Buffer*, and shake by mechanical means until disintegrated. Add 100 mL of methanol, and sonicate for about 5 min. Allow to cool to room temperature, and then dilute with *Diluent* to volume. Before taking an aliquot for dilution, allow to stand until the residue settles. Transfer a volume of the clear supernatant to a 50-mL volumetric flask to obtain a final nominal concentration between 0.090 and 0.10 mg/mL of citalopram. Add 5.0 mL of *Internal standard solution*, and dilute with *Diluent* to volume. Pass a portion through a filter (PTFE) having a 0.45- $\mu$ m or finer pore size.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 45°

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu$ L

### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for citalopram related compound F and citalopram are about 1.36 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 1.5 between citalopram and citalopram related compound F

**Column efficiency:** NLT 2000 theoretical plates, calculated from the citalopram peak

**Relative standard deviation:** NMT 1.5% for the peak response ratio of citalopram to the internal standard

## Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of citalopram ( $C_{20}H_{21}FN_2O$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak response ratio of citalopram to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of citalopram to the internal standard from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of citalopram, 324.39

$M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

**Buffer:** pH 1.5 buffer (prepared by transferring 118 mL of 1 N hydrochloric acid and 82 mL of 1 N sodium hydroxide to a 1000-mL volumetric flask, diluting with water to volume, and adjusting with 1 N sodium hydroxide to a pH of 1.5)

**Medium:** *Buffer*, 800 mL, deaerated

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Standard solution:** 12  $\mu$ g/mL of USP Citalopram Hydrobromide RS in *Medium*

**Sample solution:** Sample per *Dissolution* (711). Pass through a PVDF filter having a 0.45- $\mu$ m pore size, and dilute with *Medium* as needed.

### Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** UV

**Analytical wavelength:** 239 nm

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of citalopram ( $C_{20}H_{21}FN_2O$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times D \times (M_{r1}/M_{r2}) \times (1/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 800 mL

$D$  = dilution factor of the *Sample solution*

$M_{r1}$  = molecular weight of citalopram, 324.39

$M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

$L$  = label claim of citalopram (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of citalopram ( $C_{20}H_{21}FN_2O$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

**Buffer:** 3.15 g/L of monobasic potassium phosphate and 3.60 g/L of dibasic sodium phosphate dodecahydrate ( $Na_2HPO_4 \cdot 12H_2O$ ) in water

**Mobile phase:** Methanol, acetonitrile, and *Buffer*

(38:7:55). Adjust with phosphoric acid to a pH of 6.5.

**Standard stock solution:** 0.25 mg/mL of USP

Citalopram Hydrobromide RS in *Mobile phase*

**System suitability solution:** 1  $\mu$ g/mL each of USP

Citalopram Related Compound A RS, USP Citalopram

Related Compound B RS, USP Citalopram Related Compound C RS, and USP Citalopram Related Compound E

RS in the *Standard stock solution*



**Standard solution:** 0.625 µg/mL of citalopram hydrobromide from the *Standard stock solution* in *Mobile phase*

**Sensitivity solution:** 0.05 µg/mL of citalopram hydrobromide from the *Standard solution* in *Mobile phase*

**Sample solution:** Transfer 10 Tablets to a 200-mL volumetric flask, add 25 mL of *Buffer*, and shake by mechanical means until disintegrated. Add 100 mL of a mixture of methanol and water (1:1), mix, and sonicate for about 5 min. Allow to cool, dilute with a mixture of methanol and water (1:1) to volume, and mix thoroughly. Allow the excipients to settle. Dilute with *Mobile phase*, as necessary, to a final concentration of 0.5 mg/mL of citalopram. Pass a portion of this solution through a polytetrafluoroethylene (PTFE) membrane filter having a 0.45-µm or finer pore size, and use the filtrate.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Column temperature:** 45°

**Flow rate:** 0.8 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution*, *Standard solution*, and *Sensitivity solution*

[NOTE—See *Table 1* for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 3 between citalopram related compound C and citalopram, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Signal-to-noise ratio:** NLT 3, *Sensitivity solution*

#### Analysis

**Samples:** *System suitability solution*, *Standard solution*, and *Sample solution*

Chromatograph the *System suitability solution*, and identify the components on the basis of their relative retention times given in *Table 1*.

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of the corresponding peak from the *Standard solution*

$C_S$  = concentration of citalopram hydrobromide in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of citalopram in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of citalopram, 324.39

$M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

$F$  = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound A	0.43	0.77	0.2
Citalopram related compound B	0.60	0.98	0.25
Citalopram related compound C	0.83	0.69	0.25
Citalopram	1.0	—	—

**Table 1 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Citalopram related compound E	1.32	0.91	0.1
Any other individual, unidentified impurity	—	1.0	0.2
Total impurities	—	—	0.8

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Citalopram Hydrobromide RS

USP Citalopram Related Compound A RS

1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.

$C_{20}H_{23}FN_2O_2$  342.22

USP Citalopram Related Compound B RS

1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydroisobenzofuran-5-carbonitrile oxalate.

$C_{20}H_{21}FN_2O_2 \cdot C_2H_2O_4$  430.43

USP Citalopram Related Compound C RS

3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3H)-isobenzofuranone oxalate.

$C_{20}H_{19}FN_2O_2 \cdot C_2H_2O_4$  428.42

USP Citalopram Related Compound E RS

1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydrobenzofuran-5-carbonitrile-N-oxide hydrochloride.

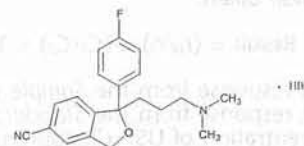
$C_{20}H_{21}FN_2O_2 \cdot HCl$  376.85

USP Citalopram Related Compound F RS

Dimethyl-(1-methyl-3,3-diphenylallyl)amine hydrochloride.

$C_{18}H_{21}N \cdot HCl$  287.83

### Citalopram Hydrobromide



$C_{20}H_{21}FN_2O \cdot HBr$  405.30

5-Isobenzofurancarboxonitrile, 1-[3-(dimethylamino)propyl]-

1-(4-fluorophenyl)-1,3-dihydro-, monohydrobromide;

1-[3-(Dimethylamino)propyl]-1-(p-fluorophenyl)-5-phthalan-carbonitrile monohydrobromide [59729-32-7].

#### DEFINITION

Citalopram Hydrobromide contains NLT 98.0% and NMT 102.0% of citalopram hydrobromide ( $C_{20}H_{21}FN_2O \cdot HBr$ ), calculated on the anhydrous basis.

#### IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

• **C. IDENTIFICATION TESTS—GENERAL, Bromide** (191)

**Sample solution:** 10 mg/mL of Citalopram Hydrobromide in water

**Acceptance criteria:** Meets the requirements of the silver nitrate precipitate test



**ASSAY****• PROCEDURE**

**Buffer:** Dissolve 1 g of sodium acetate in 800 mL of water, and add 6 mL of triethylamine. Adjust with acetic acid to a pH of 4.6, and dilute with water to 1 L.

**Mobile phase:** Acetonitrile and Buffer (20:80). The apparent pH is  $5.0 \pm 0.1$ . Make adjustments, if necessary.

**Diluent:** Methanol and water (50:50)

**System suitability solution:** 1 µg/mL each of USP Citalopram Hydrobromide RS and USP Citalopram Related Compound D RS in Diluent

**Standard solution:** 0.625 mg/mL of USP Citalopram Hydrobromide RS in Diluent

**Sample solution:** 0.625 mg/mL of Citalopram Hydrobromide in Diluent

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L7

**Column temperature:** 50°

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**Run time:** NLT 1.3 times the retention time of citalopram

**System suitability**

**Samples:** System suitability solution and Standard solution

**Suitability requirements**

**Resolution:** NLT 1.8 between citalopram related compound D and citalopram, System suitability solution

**Column efficiency:** NLT 3000 theoretical plates, Standard solution

**Tailing factor:** NMT 3.0, Standard solution

**Relative standard deviation:** NMT 2.0%, Standard solution

**Analysis**

**Samples:** Diluent, Standard solution, and Sample solution

Verify that there are no interfering peaks, using the Diluent.

Calculate the percentage of citalopram hydrobromide ( $C_{20}H_{21}FN_2O \cdot HBr$ ) in the portion of Citalopram Hydrobromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Citalopram Hydrobromide RS in the Standard solution (mg/mL)

$C_U$  = concentration of Citalopram Hydrobromide in the Sample solution (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**IMPURITIES****• RESIDUE ON IGNITION (281)**

**Analysis:** Moisten the sample with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Acceptance criteria:** NMT 0.1%

**Delete the following:****• HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1, Jan-2018)**• ORGANIC IMPURITIES, PROCEDURE 1**

[NOTE—On the basis of the synthetic route used, perform either Procedure 1 or Procedure 2. However, if the chloro and bromo analogs are potential related compounds in the synthetic route used, Procedure 2 is recommended.]

**Buffer, Mobile phase, Diluent, System suitability solution, and Sample solution:** Proceed as directed in the Assay.

**Standard solution:** 0.625 µg/mL of USP Citalopram Hydrobromide RS in Diluent

**Sensitivity solution:** 0.0625 µg/mL of USP Citalopram Hydrobromide RS in Diluent

**Chromatographic system:** Proceed as directed in the Assay, except use a Run time of 1.7 times the retention time of citalopram.

**System suitability**

**Samples:** System suitability solution and Sensitivity solution

[NOTE—See Table 1 for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 1.8 between citalopram related compound D and citalopram, System suitability solution

**Tailing factor:** 0.8–1.5 for citalopram, System suitability solution

**Relative standard deviation:** NMT 5% for citalopram, System suitability solution

**Signal-to-noise ratio:** NLT 3, Sensitivity solution

**Analysis**

**Samples:** Diluent, Standard solution, and Sample solution

Verify that there are no interfering peaks, using the Diluent.

Calculate the percentage of each impurity in the portion of Citalopram Hydrobromide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the Sample solution

$r_S$  = peak response of citalopram from the Standard solution

$C_S$  = concentration of USP Citalopram Hydrobromide RS in the Standard solution (mg/mL)

$C_U$  = concentration of Citalopram Hydrobromide in the Sample solution (mg/mL)

$M_{r1}$  = molecular weight of citalopram, 324.39

$M_{r2}$  = molecular weight of citalopram hydrobromide, 405.30

$F$  = relative response factor (see Table 1)

**Acceptance criteria:** See Table 1.

**Table 1**

Name	Relative Retention Time	Relative Response Factor <sup>a</sup>	Acceptance Criteria, NMT (%)
Citalopram ketone <sup>b</sup>	0.13	0.34	0.1
Citalopram related compound A	0.18	0.77	0.1
Citalopram open ring <sup>c</sup>	0.26	0.99	0.1
Citalopram related compound B <sup>d</sup>	0.40	0.98	0.1
Citalopram related compound C	0.67	0.69	0.1
Citalopram related compound D	0.90	1.04	0.1
Citalopram	1.0	—	—

<sup>a</sup> The relative response factors provided are for each impurity relative to citalopram (free base).

<sup>b</sup> 4-(Dimethylamino)-1-(1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl)butan-1-one.

<sup>c</sup> 4-[4-(Dimethylamino)-1-(4-fluorophenyl)-1-hydroxybutyl]-3-(hydroxymethyl)benzonitrile.

<sup>d</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydroisobenzofuran-5-carbonitrile.

<sup>e</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile-N-oxide.



Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor <sup>a</sup>	Acceptance Criteria, NMT (%)
Citalopram related compound E <sup>c</sup>	1.29	0.91	0.1
Individual unknown impurity	—	1.0	0.1
Total impurities	—	—	0.5

<sup>a</sup> The relative response factors provided are for each impurity relative to citalopram (free base).

<sup>b</sup> 4-(Dimethylamino)-1-[1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl]butan-1-one.

<sup>c</sup> 4-[4-(Dimethylamino)-1-(4-fluorophenyl)-1-hydroxybutyl]-3-(hydroxymethyl)benzonitrile.

<sup>d</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-hydroxy-1,3-dihydroisobenzofuran-5-carbonitrile.

<sup>e</sup> 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile-*N*-oxide.

#### • ORGANIC IMPURITIES, PROCEDURE 2

**Buffer:** To each L of 2.7 g/L of monobasic potassium phosphate in water prepared, add 1 mL of *N,N*-dimethyloctylamine, and adjust with phosphoric acid to a pH of 3.0.

**Solution A:** Methanol, tetrahydrofuran, and *Buffer* (24:6:70)

**Solution B:** Acetonitrile and *Buffer* (80:20)

**Mobile phase:** See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
18	100	0
40	10	90
45	10	90
46	100	0
55	100	0

**Diluent:** Acetonitrile and *Buffer* (30:70)

**System suitability solution:** 1.5 µg/mL each of USP Citalopram Hydrobromide RS, USP Citalopram Related Compound A RS, USP Citalopram Related Compound C RS, USP Citalopram Related Compound D RS, USP Citalopram Related Compound G RS, and USP Citalopram Related Compound H RS in *Diluent*

**Standard solution:** 1.5 µg/mL of USP Citalopram Hydrobromide RS in *Diluent*

**Sample solution:** 1.5 mg/mL of Citalopram Hydrobromide in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 224 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 0.8 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—See *Table 3* for the relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between citalopram and citalopram related compound D; NLT 4.0 between citalopram related compound G and citalopram related compound H

**Tailing factor:** NMT 1.5 for citalopram

**Relative standard deviation:** NMT 2.0% for citalopram

#### Analysis

**Samples:** *System suitability solution*, *Standard solution*, and *Sample solution*

Chromatograph the *System suitability solution*, and identify the components on the basis of their relative retention times, as shown in *Table 3*.

Calculate the percentage of each impurity in the portion of Citalopram Hydrobromide taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak area of each impurity from the *Sample solution*

$r_s$  = peak area of citalopram from the *Standard solution*

$C_s$  = concentration of USP Citalopram Hydrobromide RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Citalopram Hydrobromide in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 3*)

**Acceptance criteria:** See *Table 3*.

Table 3

Name	Relative Retention Time	Relative Response Factor <sup>a</sup>	Acceptance Criteria, NMT (%)
Bromide <sup>b</sup>	0.24	—	—
Citalopram related compound A	0.40	0.73	0.15
Citalopram related compound C	0.88	1.7	0.15
Citalopram	1.0	—	—
Citalopram related compound D	1.09	0.93	0.15
Citalopram related compound G	2.20	1.2	0.15
Citalopram related compound H	2.30	1.1	0.15
Individual unspecified impurity	—	1.0	0.1
Total impurities	—	—	0.75

<sup>a</sup> The relative response factors provided are for each impurity relative to citalopram hydrobromide.

<sup>b</sup> This peak is due to the counterion. It is not an impurity and should not be included in the *Total impurities*.

#### SPECIFIC TESTS

##### • OPTICAL ROTATION, *Specific Rotation* (781S)

**Sample solution:** 25 mg/mL of Citalopram Hydrobromide in methanol

**Acceptance criteria:** −0.2° to +0.2° at 20°

##### • PH (791)

**Sample solution:** 5 mg/mL of Citalopram Hydrobromide in water

**Acceptance criteria:** 5.5–6.5

##### • WATER DETERMINATION, *Method I* (921)

**Sample:** 250 mg of Citalopram Hydrobromide

**Acceptance criteria:** NMT 0.5%

##### • COMPLETENESS OF SOLUTION

**Blank:** 96% alcohol

**Sample solution:** 25 mg/mL of Citalopram Hydrobromide in 96% alcohol

**Analytical wavelength:** 410 nm

**Acceptance criteria:** Absorbance is NMT 0.040 in a 1-cm quartz cell

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers, and store at controlled room temperature.



- **LABELING:** If a procedure for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* procedure the article complies.

- **USP REFERENCE STANDARDS (11)**

USP Citalopram Hydrobromide RS

USP Citalopram Related Compound A RS

1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide.

$C_{20}H_{23}FN_2O_2$  342.41

USP Citalopram Related Compound C RS

3-(3-Dimethylaminopropyl)-3-(4-fluorophenyl)-6-cyano-1(3H)-isobenzofuranone oxalate.

$C_{20}H_{19}FN_2O_2 \cdot C_2H_2O_4$  428.42

USP Citalopram Related Compound D RS

[NOTE—May be available as a hydrochloride or a hydrobromide salt.]

1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrochloride.

$C_{19}H_{19}FN_2O \cdot HCl$  346.83

1-(4-Fluorophenyl)-1-(3-methylaminopropyl)-1,3-dihydroisobenzofuran-5-carbonitrile hydrobromide.

$C_{19}H_{19}FN_2O \cdot HBr$  391.28

USP Citalopram Related Compound G RS

3-[5-Chloro-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-N,N-dimethylpropan-1-amine hydrobromide.

$C_{19}H_{21}ClFNO \cdot HBr$  414.74

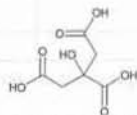
USP Citalopram Related Compound H RS

3-[5-Bromo-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-1-yl]-N,N-dimethylpropan-1-amine hydrobromide.

$C_{19}H_{21}BrFNO \cdot HBr$  459.19

## Anhydrous Citric Acid

Portions of the monograph text that are national *USP* text, and are not part of the harmonized text, are marked with symbols (♦) to signify this fact.



$C_6H_8O_7$

1,2,3-Propanetricarboxylic acid, 2-hydroxy-; Citric acid [77-92-9].

192.1

### DEFINITION

Anhydrous Citric Acid contains NLT 99.5% and NMT 100.5% of  $C_6H_8O_7$ , calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K):** Dry the substance to be examined at 105° for 2 h.

### ASSAY

- **PROCEDURE**

**Sample:** 0.550 g of Anhydrous Citric Acid; record weight accurately.

**Analysis:** Dissolve the *Sample* in 50 mL of water. Add 0.5 mL of phenolphthalein TS. Titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 64.03 mg of  $C_6H_8O_7$ .

**Acceptance criteria:** 99.5%–100.5% on the anhydrous basis

### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%, determined on 1.0 g

### Delete the following:

- **♦HEAVY METALS (231):** NMT 10 µg/g♦♦ (Official 1-Jan-2018)

- **SULFATE**

**Standard sulfate solution A:** 1.81 mg/mL of potassium sulfate in 30% alcohol. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with 30% alcohol to volume, and mix. This solution contains 10 µg/mL of sulfate.

**Standard sulfate solution B:** 1.81 mg/mL of potassium sulfate in water. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 10 µg/mL of sulfate.

**Sample stock solution:** 66.7 mg/mL of Anhydrous Citric Acid

**Sample solution:** To 4.5 mL of *Standard sulfate solution A*, add 3 mL of a barium chloride solution (1 in 4), shake, and allow to stand for 1 min. To 2.5 mL of the resulting suspension, add 15 mL of the *Sample stock solution* and 0.5 mL of 5 N acetic acid, and mix.

**Standard solution:** Prepare as directed for the *Sample solution*, except use 15 mL of *Standard sulfate solution B* instead of the *Sample stock solution*.

### Analysis

**Samples:** *Sample solution* and *Standard solution*

**Acceptance criteria:** Any turbidity produced in the *Sample solution* after 5 min standing is not greater than that produced in the *Standard solution* (0.015%).

- **LIMIT OF ALUMINUM** (where it is labeled as intended for use in dialysis)

**Standard aluminum solution:** To 352 mg of aluminum potassium sulfate in a 100-mL volumetric flask, add a few mL of water, swirl to dissolve, add 10 mL of diluted sulfuric acid, dilute with water to volume, and mix. Immediately before use, dilute 1.0 mL of this solution with water to 100.0 mL.

**pH 6.0 acetate buffer:** Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, dilute with water to 250 mL, and mix.

**Sample solution:** Dissolve 20.0 g of Anhydrous Citric Acid in 100 mL of water, and add 10 mL of pH 6.0 acetate buffer. Extract this solution with successive portions of 20, 20, and 10 mL of a 0.5% solution of 8-hydroxyquinoline in chloroform, combining the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume, and mix.

**Standard solution:** Prepare a mixture of 2.0 mL of *Standard aluminum solution*, 10 mL of pH 6.0 acetate buffer, and 98 mL of water. Extract this mixture as described for the *Sample solution*, dilute the combined extracts with chloroform to volume, and mix.

**Blank solution:** Prepare a mixture of 10 mL of pH 6.0 acetate buffer and 100 mL of water. Extract this mixture as described for *Sample solution*, dilute the combined extracts with chloroform to volume, and mix.

### Fluorometric conditions

**Excitation wavelength:** 392 nm

**Emission wavelength:** 518 nm

### Analysis

**Samples:** *Sample solution* and *Standard solution*

Determine the fluorescence intensities of the *Samples* in a fluorometer set as directed under *Fluorometric conditions*, using the *Blank solution* to set the instrument to zero.

**Acceptance criteria:** The fluorescence of the *Sample solution* does not exceed that of the *Standard solution* (0.2 ppm).

- **LIMIT OF OXALIC ACID**

**Sample stock solution:** 0.80 g of Anhydrous Citric Acid in 4 mL of water

**Sample solution:** To the *Sample stock solution* add 3 mL of hydrochloric acid and 1 g of granular zinc, boil for 1



min, and allow to stand for 2 min. Transfer the supernatant to a test tube containing 0.25 mL of a phenylhydrazine hydrochloride solution (1 in 100), and heat to boiling. Cool rapidly, transfer to a graduated cylinder, and add an equal volume of hydrochloric acid and 0.25 mL of a potassium ferricyanide solution (1 in 20). Shake, and allow to stand for 30 min.

**Standard solution:** Prepare as directed for the *Sample solution*, except use 4 mL of 0.10 mg/mL oxalic acid solution, equivalent to 0.0714 mg/mL of anhydrous oxalic acid, instead of the *Sample stock solution*. [NOTE—Prepare concomitantly with the *Sample solution*.]

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

**Acceptance criteria:** Any pink color produced in the *Sample solution* is not more intense than that produced in the *Standard solution* (0.036%).

### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** The level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Anhydrous Citric Acid is used can be met. Where the label states that Anhydrous Citric Acid must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Anhydrous Citric Acid is used can be met.♦

#### • CLARITY OF SOLUTION

[NOTE—The *Sample solution* is to be compared to *Standard suspension A* in diffused daylight 5 min after preparation of *Standard suspension A*.]

**Hydrazine sulfate solution:** 10 mg/mL of hydrazine sulfate in water. Allow to stand for 4–6 h before use.

**Methenamine solution:** Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

**Primary opalescent suspension:** Transfer 25.0 mL of *Hydrazine sulfate solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. [NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.]

**Opalescence standard:** Dilute 15.0 mL of *Primary opalescent suspension* with water to 1000 mL. [NOTE—This suspension should not be used beyond 24 h after preparation.]

**Standard suspension A:** Dilute 5.0 mL of *Opalescence standard* with 95 mL of water.

**Standard suspension B:** Dilute 10.0 mL of *Opalescence standard* with 90 mL of water.

**Sample solution:** 200 mg/mL of Anhydrous Citric Acid in water

#### Analysis

**Samples:** *Standard suspension A*, *Standard suspension B*, water, and *Sample solution*

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension A*, *Standard suspension B*, and water to separate matching test tubes. Compare the *Sample solution*, *Standard suspension A*, *Standard suspension B*, and water in diffused daylight, viewing vertically against a black background (see *Nephelometry, Turbidimetry, and Visual Comparison (855), Visual Comparison*). [NOTE—The diffusion of light must be such that *Standard suspension A* can readily be distinguished from water, and that *Standard suspension B* can readily be distinguished from *Standard suspension A*.]

**Acceptance criteria:** The *Sample solution* shows the same clarity as that of water or its opalescence is not more pronounced than *Standard suspension A*.

#### • COLOR OF SOLUTION

**Standard stock solution A:** Ferric chloride CS, cobaltous chloride CS, and dilute hydrochloric acid (10 g/L) (2.4: 0.6: 7.0)

**Standard stock solution B:** Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (2.4: 1.0: 0.4: 6.2)

**Standard stock solution C:** Ferric chloride CS, cobaltous chloride CS, and cupric sulfate CS (9.6: 0.2: 0.2)

[NOTE—Prepare the *Standard solutions* immediately before use.]

**Standard solution A:** Dilute 2.5 mL of *Standard stock solution A* with dilute hydrochloric acid (10 g/L) to 100 mL.

**Standard solution B:** Dilute 2.5 mL of *Standard stock solution B* with dilute hydrochloric acid (10 g/L) to 100 mL.

**Standard solution C:** Dilute 0.75 mL of *Standard stock solution C* with dilute hydrochloric acid (10 g/L) to 100 mL.

**Sample solution:** Prepare as directed in the test for *Clarity of Solution*.

#### Analysis 1

**Samples:** Water and *Sample solution*

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer water to a separate matching test tube. Compare the *Sample solution* and water in diffused daylight, viewing vertically against a white background (see *Nephelometry, Turbidimetry, and Visual Comparison (855), Visual Comparison*).

**Acceptance criteria 1:** The *Sample solution* is not more intensely colored than water. If more intensely colored, follow *Analysis 2*.

#### Analysis 2

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Transfer a sufficient portion of *Standard solution A*, *Standard solution B*, and *Standard solution C* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Compare the *Sample solution* from *Analysis 1* to *Standard solution A*, *Standard solution B*, and *Standard solution C* in diffused daylight, viewing vertically against a white background (see *Nephelometry, Turbidimetry, and Visual Comparison (855), Visual Comparison*).

**Acceptance criteria 2:** The *Sample solution* is not more intensely colored than *Standard solutions A*, *B*, and *C*.

#### • READILY CARBONIZABLE SUBSTANCES

**Sample:** 1.0 g of powdered Anhydrous Citric Acid

**Analysis:** Transfer the *Sample* to a 22-mm × 175-mm test tube previously rinsed with 10 mL of sulfuric acid and allowed to drain for 10 min. Add 10 mL of sulfuric acid, agitate until solution is complete, and immerse in a water bath at 90 ± 1° for 60 ± 0.5 min, keeping the level of the acid below the level of the water during the entire period. Cool the tube in running water, and transfer the acid to a color-comparison tube.

**Acceptance criteria:** The color of the acid is not darker than that of a similar volume of *Matching Fluid K* (see *Color and Achromicity (631)*) in a matching tube, the tubes being observed vertically against a white background.

- **STERILITY TESTS (71):** Where the label states that Anhydrous Citric Acid is sterile, it meets the requirements for *Sterility Tests (71)* in the relevant dosage form monograph(s) in which Anhydrous Citric Acid is used.♦



• **WATER DETERMINATION, Method I (921)**

Sample: 2.0 g of Anhydrous Citric Acid

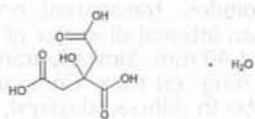
Acceptance criteria: NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. No storage requirements specified.
- **LABELING:** Where it is intended for use in dialysis solutions, it is so labeled. Where Anhydrous Citric Acid must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled. Where Anhydrous Citric Acid is sterile, it is so labeled.
- **USP REFERENCE STANDARDS (11)**  
USP Citric Acid RS  
USP Endotoxin RS.

## Citric Acid Monohydrate

Portions of the monograph text that are national USP text, and are not part of the harmonized text, are marked with symbols (♦) to specify this fact.



$C_6H_8O_7 \cdot H_2O$  210.14  
1,2,3-Propanetricarboxylic acid, 2-hydroxy-, monohydrate  
[5949-29-1].

**DEFINITION**

Citric Acid Monohydrate contains one molecule of water of hydration. It contains NLT 99.5% and NMT 100.5% of  $C_6H_8O_7$ , calculated on the anhydrous basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K):** Dry the substance to be examined at 105° for 2 h.

**ASSAY**

• **PROCEDURE**

Sample: 0.550 g of Citric Acid Monohydrate. Record the weight accurately.

Analysis: Dissolve the Sample in 50 mL of water, and add 0.5 mL of phenolphthalein TS. Titrate with 1 N sodium hydroxide VS. Each mL of 1 N sodium hydroxide is equivalent to 64.03 mg of  $C_6H_8O_7$ .

Acceptance criteria: 99.5%–100.5% on the anhydrous basis

**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 0.1%, determined on 1.0 g

Delete the following:

- ♦**HEAVY METALS (231):** NMT 10 µg/g♦♦ (Official 1-Jan-2018)

• **SULFATE**

Standard sulfate solution A: 1.81 mg/mL of potassium sulfate in 30% alcohol. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with 30% alcohol to volume, and mix. This solution contains 10 µg/mL of sulfate.

Standard sulfate solution B: 1.81 mg/mL of potassium sulfate. Immediately before use, transfer 10.0 mL of this solution to a 1000-mL volumetric flask, dilute with water to volume, and mix. This solution contains 10 µg/mL of sulfate.

Sample stock solution: 66.7 mg/mL of Citric Acid Monohydrate

Sample solution: To 4.5 mL of Standard sulfate solution A, add 3 mL of a barium chloride solution (1 in 4), shake, and allow to stand for 1 min. To 2.5 mL of the resulting suspension add 15 mL of the Sample stock solution and 0.5 mL of 5 N acetic acid, and mix.

Standard solution: Prepare as directed in the Sample solution, except use 15 mL of Standard sulfate solution B instead of the Sample stock solution.

**Analysis**

Samples: Sample solution and Standard solution

Acceptance criteria: Any turbidity produced in the Sample solution after 5 min standing is not greater than that produced in the Standard solution (0.015%).

- **LIMIT OF ALUMINUM** (where it is labeled as intended for use in dialysis)

Standard aluminum solution: To 352 mg of aluminum potassium sulfate in a 100-mL volumetric flask, add a few mL of water, swirl to dissolve, add 10 mL of diluted sulfuric acid, and dilute with water to volume. Immediately before use, dilute 1.0 mL of this solution with water to 100.0 mL.

pH 6.0 acetate buffer: Dissolve 50 g of ammonium acetate in 150 mL of water, adjust with glacial acetic acid to a pH of 6.0, dilute with water to 250 mL, and mix.

Sample solution: Dissolve 20.0 g of Citric Acid Monohydrate in 100 mL of water, and add 10 mL of pH 6.0 acetate buffer. Extract this solution with successive portions of 20, 20, and 10 mL of a 0.5% solution of 8-hydroxyquinoline in chloroform, combining the chloroform extracts in a 50-mL volumetric flask. Dilute the combined extracts with chloroform to volume, and mix.

Standard solution: Prepare a mixture of 2.0 mL of Standard aluminum solution, 10 mL of pH 6.0 acetate buffer, and 98 mL of water. Extract this mixture as described for the Sample solution, dilute the combined extracts with chloroform to volume, and mix.

Blank solution: Prepare a mixture of 10 mL of pH 6.0 acetate buffer and 100 mL of water. Extract this mixture as described for the Sample solution, dilute the combined extracts with chloroform to volume, and mix.

**Fluorometric conditions**

Excitation wavelength: 392 nm

Emission wavelength: 518 nm

**Analysis**

Samples: Sample solution and Standard solution

Determine the fluorescence intensities of the Samples in a fluorometer set as directed under Fluorometric conditions, using the Blank solution to set the instrument to zero.

Acceptance criteria: The fluorescence of the Sample solution does not exceed that of the Standard solution (0.2 ppm).

- **LIMIT OF OXALIC ACID**

Sample stock solution: 0.80 g of Citric Acid Monohydrate in 4 mL of water

Sample solution: To the Sample stock solution add 3 mL of hydrochloric acid and 1 g of granular zinc, boil for 1 min, and allow to stand for 2 min. Transfer the supernatant to a test tube containing 0.25 mL of a phenylhydrazine hydrochloride solution (1 in 100), and heat to boiling. Cool rapidly, transfer to a graduated cylinder, and add an equal volume of hydrochloric acid and 0.25 mL of a potassium ferricyanide solution (1 in 20). Shake and allow to stand for 30 min.

Standard solution: Prepare as directed for the Sample solution, except use 4 mL of 0.10 mg/mL oxalic acid solution, equivalent to 0.0714 mg/mL of anhydrous oxalic acid, instead of the Sample stock solution. [NOTE—Prepare concomitantly with the Sample solution.]



**Analysis**

**Samples:** *Sample solution* and *Standard solution*

**Acceptance criteria:** Any pink color produced in the *Sample solution* is not more intense than that produced in the *Standard solution* (0.036%).

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST (85):** The level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Citric Acid Monohydrate is used can be met. Where the label states that Citric Acid Monohydrate must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement in the relevant dosage form monograph(s) in which Citric Acid Monohydrate is used can be met.♦

- **CLARITY OF SOLUTION**

[NOTE—The *Sample solution* is to be compared to *Standard suspension A* in diffused daylight 5 min after preparation of *Standard suspension A*.]

**Hydrazine sulfate solution:** 10 mg/mL of hydrazine sulfate in water. Allow to stand for 4–6 h before use.

**Methenamine solution:** Transfer 2.5 g of methenamine to a 100-mL glass-stoppered flask, add 25.0 mL of water, insert the glass stopper, and mix to dissolve.

**Primary opalescent suspension:** Transfer 25.0 mL of *Hydrazine sulfate solution* to the *Methenamine solution* in the 100-mL glass-stoppered flask. Mix, and allow to stand for 24 h. [NOTE—This suspension is stable for 2 months, provided it is stored in a glass container free from surface defects. The suspension must not adhere to the glass and must be well mixed before use.]

**Opalescence standard:** Dilute 15.0 mL of *Primary opalescent suspension* with water to 1000 mL. [NOTE—This suspension should not be used beyond 24 h after preparation.]

**Standard suspension A:** Dilute 5.0 mL of *Opalescence standard* with 95 mL of water.

**Standard suspension B:** Dilute 10.0 mL of *Opalescence standard* with 90 mL of water.

**Sample solution:** 200 mg/mL of Citric Acid Monohydrate in water

**Analysis**

**Samples:** *Standard suspension A*, *Standard suspension B*, water, and *Sample solution*

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer portions of *Standard suspension A*, *Standard suspension B*, and water to separate matching test tubes. Compare the *Sample solution*, *Standard suspension A*, *Standard suspension B*, and water in diffused daylight, viewing vertically against a black background (see *Nephelometry*, *Turbidimetry*, and *Visual Comparison* (855), *Visual Comparison*). [NOTE—The diffusion of light must be such that *Standard suspension A* can readily be distinguished from water, and that *Standard suspension B* can readily be distinguished from *Standard suspension A*.]

**Acceptance criteria:** The *Sample solution* shows the same clarity as that of water or its opalescence is not more pronounced than *Standard suspension A*.

- **COLOR OF SOLUTION**

**Standard stock solution A:** Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (2.4: 0.6: 0: 7.0)

**Standard stock solution B:** Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (2.4: 1.0: 0.4: 6.2)

**Standard stock solution C:** Ferric chloride CS, cobaltous chloride CS, cupric sulfate CS, and dilute hydrochloric acid (10 g/L) (9.6: 0.2: 0.2: 0)

[NOTE—Prepare the *Standard solutions* immediately before use.]

**Standard solution A:** Transfer 2.5 mL of *Standard stock solution A*, and dilute with dilute hydrochloric acid (10 g/L) to 100 mL.

**Standard solution B:** Transfer 2.5 mL of *Standard stock solution B*, and dilute with dilute hydrochloric acid (10 g/L) to 100 mL.

**Standard solution C:** Transfer 0.75 mL of *Standard stock solution C*, and dilute with dilute hydrochloric acid (10 g/L) to 100 mL.

**Sample solution:** Prepare as directed in the test for *Clarity of Solution*.

**Analysis 1**

**Samples:** Water and *Sample solution*

Transfer a sufficient portion of the *Sample solution* to a test tube of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Similarly transfer water to a separate matching test tube. Compare the *Sample solution* and water in diffused daylight, viewing vertically against a white background (see *Nephelometry*, *Turbidimetry*, and *Visual Comparison* (855), *Visual Comparison*).

**Acceptance criteria 1:** The *Sample solution* is not more intensely colored than water. If more intensely colored, follow *Analysis 2*.

**Analysis 2**

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Transfer a sufficient portion of *Standard solution A*, *Standard solution B*, and *Standard solution C* to separate test tubes of colorless, transparent, neutral glass with a flat base and an internal diameter of 15–25 mm to obtain a depth of 40 mm. Compare the *Sample solution* from *Analysis 1* to *Standard solution A*, *Standard solution B*, and *Standard solution C* in diffused daylight, viewing vertically against a white background (see *Nephelometry*, *Turbidimetry*, and *Visual Comparison* (855), *Visual Comparison*).

**Acceptance criteria 2:** The *Sample solution* is not more intensely colored than *Standard solutions A*, *B*, and *C*.

- **READILY CARBONIZABLE SUBSTANCES**

**Sample:** 1.0 g powdered Citric Acid Monohydrate

**Analysis:** Transfer the *Sample* to a 22-mm × 175-mm test tube previously rinsed with 10 mL of sulfuric acid and allowed to drain for 10 min. Add 10 mL of sulfuric acid, agitate until solution is complete, and immerse in a water bath at 90 ± 1° for 60 ± 0.5 min, keeping the level of the acid below the level of the water during the entire period. Cool the tube in running water, and transfer the acid to a color-comparison tube.

**Acceptance criteria:** The color of the acid is not darker than that of a similar volume of *Matching Fluid K* (see *Color and Achromicity* (631)) in a matching tube, the tubes being observed vertically against a white background.

- **STERILITY TESTS (71):** Where the label states that Citric Acid Monohydrate is sterile, it meets the requirements for *Sterility Tests* (71) in the relevant dosage form monograph(s) in which Citric Acid Monohydrate is used.♦

- **WATER DETERMINATION, Method I (921)**

**Sample:** 0.5 g of Citric Acid Monohydrate

**Acceptance criteria:** 7.5%–9.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. No storage requirements specified.
- **LABELING:** Where it is intended for use in dialysis solutions, it is so labeled. Where Citric Acid Monohydrate must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled. Where Citric Acid Monohydrate is sterile, it is so labeled.



• **USP REFERENCE STANDARDS** (11)

USP Citric Acid RS  
USP Endotoxin RS

## Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation

### DEFINITION

Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation is a sterile solution of Citric Acid, Magnesium Oxide, and Sodium Carbonate in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amounts of citric acid ( $C_6H_8O_7 \cdot H_2O$ ), magnesium oxide (MgO), and sodium carbonate ( $Na_2CO_3$ ).

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL**, Sodium (191) and Magnesium (191)

- **B.**

**Sample solution:** 10 mL of Irrigation

**Analysis:** Add 1 mL of mercuric sulfate TS to the *Sample solution*, heat to boiling, and add a few drops of potassium permanganate TS.

**Acceptance criteria:** A white precipitate is formed.

### ASSAY

- **CITRIC ACID**

**Mobile phase, Standard preparation 1, and Chromatographic system:** Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345).

**Assay preparation for citric acid/citrate assay:** Nominally 20 µg/mL of citrate from Irrigation in 1 mM of sodium hydroxide prepared as follows. Transfer a suitable volume of Irrigation to an appropriately sized volumetric flask, and proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345), *Assay Preparation for Citric Acid/Citrate Assay*.

#### Analysis

**Samples:** *Standard preparation 1* and *Assay preparation for citric acid/citrate assay*

Proceed as directed in *Assay for Citric Acid/Citrate and Phosphate* (345), *Procedure*.

Calculate the percentage of the labeled amount of citric acid monohydrate ( $C_6H_8O_7 \cdot H_2O$ ) in the portion of Irrigation taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak response of citrate from the *Assay preparation for citric acid/citrate assay*

$r_S$  = peak response of citrate from *Standard preparation 1*

$C_S$  = concentration of *Standard preparation 1* (µg/mL)

$C_U$  = nominal concentration of citric acid monohydrate in the *Assay preparation for citric acid/citrate assay* (µg/mL)

$M_{r1}$  = molecular weight of citric acid monohydrate, 210.14

$M_{r2}$  = molecular weight of citrate, 189.10

**Acceptance criteria:** 95.0%–105.0%

- **MAGNESIUM OXIDE**

**Sample solution:** A volume of Irrigation, nominally equivalent to 40 mg of magnesium oxide

**Analysis:** Transfer the *Sample solution* to a beaker containing 130 mL of water heated to  $75^\circ \pm 5^\circ$ , and add 4 mL of ammonium chloride TS and then 5 mL of ammonium hydroxide. Mix, and add slowly, with stirring, 8 mL of 8-hydroxyquinoline TS. After allowing to stand for 30 min at  $75^\circ$ , filter through a sintered-glass crucible, previously dried and weighed. Wash the precipitate with 50 mL of a warm mixture of water and 6 N am-

monium hydroxide (45:5), followed by 50 mL of cool water. Dry the crucible and contents at  $105^\circ$  for 3 h, cool, and weigh.

Determine the equivalent of magnesium oxide (MgO) in the portion of Irrigation taken by multiplying the weight of the  $C_{18}H_{12}MgN_2O_2 \cdot 2H_2O$  so obtained by 0.1156 (mg of MgO).

Calculate the percentage of the labeled amount of magnesium oxide (MgO) in the portion of Irrigation taken.

**Acceptance criteria:** 95.0%–105.0%

- **SODIUM CARBONATE**

**Sodium chloride stock solution:** 4.75 mg/mL of sodium chloride, previously dried at  $105^\circ$  for 2 h, in water

**Internal standard solution:** 0.636 mg/mL of lithium chloride in water

**Standard solution:** 0.0475 mg/mL of sodium chloride and 0.6296 mg/mL of lithium chloride prepared from an appropriate mixture of *Sodium chloride stock solution* and *Internal standard solution*

**Sample stock solution:** Nominally equivalent to 4.4 mg/mL of sodium carbonate from Irrigation diluted with water

**Sample solution:** 0.044 mg/mL of sodium carbonate and 0.6296 mg/mL of lithium chloride prepared from an appropriate mixture of *Sample stock solution* and *Internal standard solution*

#### Instrumental conditions

**Mode:** Flame photometer

**Analytical wavelengths:** 591 and 671 nm

#### Analysis

**Samples:** *Internal standard solution*, *Standard solution*, and *Sample solution*

Concomitantly determine the emittances of the *Standard solution* and the *Sample solution*, adjusting the instrument with *Internal standard solution* to zero emittance.

Calculate the percentage of the labeled amount of sodium carbonate ( $Na_2CO_3$ ) in the portion of Irrigation taken:

$$\text{Result} = (r_{U,591}/r_{U,671}) \times (r_{S,671}/r_{S,591}) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_{U,591}$  = emittance reading from the *Sample solution* at 591 nm

$r_{U,671}$  = emittance reading from the *Sample solution* at 671 nm

$r_{S,671}$  = emittance reading from the *Standard solution* at 671 nm

$r_{S,591}$  = emittance reading from the *Standard solution* at 591 nm

$C_S$  = concentration of sodium chloride in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of sodium carbonate in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of sodium carbonate, 105.99

$M_{r2}$  = two times the molecular weight of sodium chloride, 116.88

**Acceptance criteria:** 95.0%–105.0%

### SPECIFIC TESTS

- **pH (791):** 3.8–4.2

- **BACTERIAL ENDOTOXINS TEST (85):** It contains not more than 2.80 USP Endotoxin Units per mL.

- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1), except that the container may be designed to empty rapidly and may exceed 1000 mL in capacity.

### ADDITIONAL REQUIREMENTS

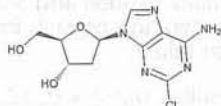
- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type I or Type II glass.



• **USP REFERENCE STANDARDS** (11)

USP Citric Acid RS  
USP Endotoxin RS

## Cladribine



$C_{10}H_{12}ClN_5O_3$  285.69  
Adenosine, 2-chloro-2'-deoxy-;  
2-Chloro-2'-deoxyadenosine [4291-63-8].

### DEFINITION

Cladribine contains NLT 98.0% and NMT 102.0% of cladribine ( $C_{10}H_{12}ClN_5O_3$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** Dissolve 9.96 g of triethylamine phosphate in 500 mL of water. Add another 500 mL of water, and adjust with potassium hydroxide to a pH of 6.1.

**Diluent:** Methanol and water (10:90)

**Mobile phase:** Methanol and *Buffer* (22:78)

**System suitability solution:** 0.02 mg/mL each of USP Cladribine RS and USP Cladribine Related Compound A RS in *Diluent*

**Standard solution:** 0.5 mg/mL of USP Cladribine RS in *Diluent*

**Sample solution:** 0.5 mg/mL of Cladribine in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between the cladribine and cladribine related compound A peaks, *System suitability solution*

**Tailing factor:** NMT 2.0 for the cladribine peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cladribine ( $C_{10}H_{12}ClN_5O_3$ ) in the portion of Cladribine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Cladribine RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Cladribine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

### Delete the following:

- **HEAVY METALS, Method II** (231): NMT 20 ppm (Official 1, Jan-2018)

### • ORGANIC IMPURITIES

**Buffer, Diluent, Mobile phase, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability**

**Sample:** *System suitability solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between cladribine and cladribine related compound A

**Tailing factor:** NMT 2.0 for the cladribine peak

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Cladribine taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each individual impurity

$r_T$  = sum of the responses of all the peaks

Acceptance criteria: See *Table 1*.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,6-Diaminopurine-2'-deoxyribose	0.41	0.20
2'-Deoxyadenosine	0.47	0.20
2-Chloroadenine	0.60	0.20
Cladribine related compound A	0.91	0.20
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation** (781S)

**Sample solution:** 10 mg/mL in dimethylformamide

**Acceptance criteria:**  $-17.0^\circ$  to  $-21.0^\circ$

- **WATER DETERMINATION, Method I** (921): NMT 4.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, and protect from light. Store between  $2^\circ$  and  $8^\circ$ .

- **USP REFERENCE STANDARDS** (11)

USP Cladribine RS

USP Cladribine Related Compound A RS

2-Methoxy-2'-deoxyadenosine.

$C_{11}H_{15}N_5O_4$  281.27

## Cladribine Injection

### DEFINITION

Cladribine Injection is a clear, colorless, sterile, preservative-free, isotonic solution. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cladribine ( $C_{10}H_{12}ClN_5O_3$ ).



**IDENTIFICATION**• **A. ULTRAVIOLET ABSORPTION** (197U)

**Sample solution:** 0.05 mg/mL of cladribine in water  
**Acceptance criteria:** Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** Dissolve 9.96 g of triethylamine phosphate, accurately weighed, in 500 mL of water, and add another 500 mL of water. Adjust with potassium hydroxide to a pH of 6.1.

[NOTE—Alternatively, dissolve 13.5 mL of triethylamine in 1 L of water, and adjust with phosphoric acid to a pH of 6.1.]

**Mobile phase:** Methanol and *Buffer* (22:78)

**Diluent:** Methanol and water (10:90)

**System suitability solution:** 0.02 mg/mL each of USP Cladribine RS and USP Cladribine Related Compound A RS in *Diluent*

**Standard solution:** 0.5 mg/mL of USP Cladribine RS in *Diluent*

**Sample solution:** Nominally, equivalent to 0.5 mg/mL of cladribine in *Diluent* from Injection

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 265 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between cladribine and cladribine related compound A, *System suitability solution*

**Tailing factor:** NMT 2.0 for the cladribine peak, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of the labeled amount of cladribine (C<sub>10</sub>H<sub>12</sub>ClN<sub>5</sub>O<sub>3</sub>) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cladribine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cladribine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**IMPURITIES**• **ORGANIC IMPURITIES**

**Buffer, Mobile phase, Diluent, System suitability solution, and Sample solution:** Proceed as directed in the *Assay*.

**Standard solution:** 0.01 mg/mL of USP Cladribine RS in *Diluent*

**Chromatographic system:** Proceed as directed in the *Assay*. In addition, the run time is NLT 2.5 times of the retention time of the cladribine peak for the *Sample solution*.

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.5 between cladribine and cladribine related compound A, *System suitability solution*

**Tailing factor:** NMT 2.0 for the cladribine peak, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of cladribine from the *Standard solution*

$C_S$  = concentration of USP Cladribine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cladribine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 1. Disregard any impurity peaks less than 0.1%.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
2,6-Diaminopurine-2'-deoxyriboside	0.41	0.2
2'-Deoxyadenosine	0.47	0.2
2-Chloroadenine	0.60	0.5
Cladribine related compound A <sup>a</sup>	0.91	0.2
Cladribine	1.0	—
Any individual, unspecified impurity	—	0.2
Total impurities	—	2.0

<sup>a</sup> 2-Methoxy-2'-deoxyadenosine.

**SPECIFIC TESTS**

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 55 USP Endotoxin Units/mg of cladribine
- **STERILITY TESTS** (71): It meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **PH** (791): 5.5–8.0
- **OSMOLALITY AND OSMOLARITY, Osmolality** (785): 250–370 mOsmol/kg
- **PARTICULATE MATTER IN INJECTIONS** (788): It meets the requirements for small-volume injections.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* (1).

**ADDITIONAL REQUIREMENTS**

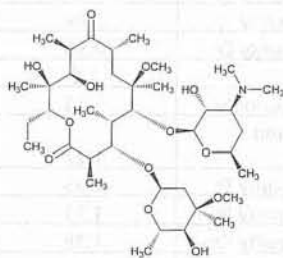
- **PACKAGING AND STORAGE:** Preserve in single-use clear flint glass vials. Store refrigerated at 2°–8°C. Protect from light.
- **LABELING:** Label it to indicate that it is to be diluted with 0.9% Sodium Chloride Injection USP for the single daily dose and to be diluted with bacteriostatic 0.9% Sodium Chloride Injection USP (0.9% benzyl alcohol preserved) to prepare the 7-day infusion solution.



• **USP REFERENCE STANDARDS** (11)

USP Cladribine RS  
USP Cladribine Related Compound A RS  
2-Methoxy-2'-deoxyadenosine,  
 $C_{11}H_{15}N_5O_4$  281.27  
USP Endotoxin RS

## Clarithromycin



$C_{38}H_{69}NO_{13}$  747.95  
Erythromycin, 6-O-methyl-;  
6-O-Methylerythromycin [81103-11-9].

### DEFINITION

Clarithromycin contains NLT 96.0% and NMT 102.0% of clarithromycin ( $C_{38}H_{69}NO_{13}$ ), calculated on the anhydrous basis.

### IDENTIFICATION

• **A. INFRARED ABSORPTION** (197K)

### ASSAY

• **PROCEDURE**

Solution A, Solution B, Mobile phase, Diluent, Standard solution 1, Standard solution 2, Standard solution 4, Sample solution, and Chromatographic system: Proceed as directed in *Organic Impurities*.

#### System suitability

Samples: Standard solution 1, Standard solution 2, and Standard solution 4

[NOTE—See the relative retention times in Table 2. The typical retention time for clarithromycin is about 11 min.]

#### Suitability requirements

Tailing factor: NMT 1.7, Standard solution 2

Peak-to-valley ratio: NLT 3.0, Standard solution 4

The Peak-to-valley ratio is calculated as follows:

$$\text{Result} = H_p/H_v$$

$H_p$  = height above the baseline of the clarithromycin impurity D peak, Standard solution 4

$H_v$  = height above the baseline of the lowest point of the curve separating the clarithromycin impurity D peak from the clarithromycin peak, Standard solution 4

Relative standard deviation: NMT 1.5%, Standard solution 1

#### Analysis

Samples: Standard solution 1 and Sample solution

Calculate the percentage of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) in the portion of Clarithromycin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak area response from the Sample solution

$r_s$  = peak area response from Standard solution 1

$C_s$  = concentration of USP Clarithromycin RS in Standard solution 1 (mg/mL)

$C_u$  = concentration of Clarithromycin in the Sample solution (mg/mL)

Acceptance criteria: 96.0%–102.0% on the anhydrous basis

### IMPURITIES

• **RESIDUE ON IGNITION** (281)

Sample: 0.5 g

Acceptance criteria: NMT 0.2%

### Delete the following:

• **HEAVY METALS**

Diluent: 85% v/v dioxane in water

Lead nitrate stock solution: Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid. Dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.

Sample solution: 50 mg/mL in Diluent. Transfer 12 mL of this solution to a color-comparison tube.

Standard solution: 1 ppm of Pb, prepared by diluting Lead nitrate stock solution with Diluent. Add 10 mL of this solution and 2 mL of the Sample solution to a color-comparison tube.

Blank: Add 10 mL of Diluent and 2 mL of the Sample solution to a color-comparison tube.

#### Analysis

Samples: Sample solution, Standard solution, and Blank  
To each of the three tubes add 2 mL of pH 3.5 acetate buffer, mix, then add 1.2 mL of thioacetamide–glycerin base TS, and mix.

Acceptance criteria: Compared to the Blank, the Standard solution shows a slight brown color. After 2 min, any brown color in the Sample solution is not more intense than that in the Standard solution (NMT 20 µg/g).

• (Official 1-Jan-2018)

• **ORGANIC IMPURITIES**

Solution A: 4.76 g/L of monobasic potassium phosphate. Adjust with dilute phosphoric acid (1 in 10) or potassium hydroxide (45% w/v) to a pH of 4.4. Pass this solution through a C18 filtration kit.

Solution B: Acetonitrile

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
32	40	60
34	40	60
36	75	25
42	75	25

Diluent: Acetonitrile and water (1:1)

Standard solution 1: 1.5 mg/mL of USP Clarithromycin RS in acetonitrile and water (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume.

Standard solution 2: 75 µg/mL of USP Clarithromycin RS from Standard solution 1 in Diluent

Standard solution 3: 7.5 µg/mL of USP Clarithromycin RS from Standard solution 2 in Diluent

Standard solution 4: 1.5 mg/mL of USP Clarithromycin Identity RS in acetonitrile and water (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume.

Sample solution: 1.5 mg/mL of Clarithromycin in acetonitrile and water (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume.



**Chromatographic system**

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 205 nm

Column: 4.6-mm × 10-cm; packing L1

Column temperature: 40°

Flow rate: 1.1 mL/min

Injection volume: 10 µL

**System suitability****Samples:** Standard solution 2 and Standard solution 4

[NOTE—See the relative retention times in Table 2. The typical retention time for clarithromycin is about 11 min.]

**Suitability requirements****Tailing factor:** NMT 1.7, Standard solution 2**Peak-to-valley ratio:** NLT 3.0, Standard solution 4

The Peak-to-valley ratio is calculated as follows:

$$\text{Result} = H_p/H_v$$

$H_p$  = height above the baseline of the clarithromycin impurity D peak, Standard solution 4

$H_v$  = height above the baseline of the lowest point of the curve separating the clarithromycin impurity D peak from the clarithromycin peak, Standard solution 4

**Analysis****Samples:** Diluent, Standard solution 2, Standard solution 3, Standard solution 4, and Sample solution

Calculate the percentage of each impurity in the portion of Clarithromycin taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of any individual impurity from the Sample solution

$r_s$  = peak response of clarithromycin from Standard solution 3

$C_s$  = concentration of USP Clarithromycin RS in Standard solution 3 (mg/mL)

$C_u$  = concentration of Clarithromycin in the Sample solution (mg/mL)

$F$  = relative response factor (see Table 2)

**Acceptance criteria:** See Table 2. The reporting threshold is 0.1%. Disregard the peaks eluting before impurity I and after impurity H.

**Any individual impurity:** NMT 1.0%. NMT four impurities exceed 0.4%.

**Total impurities:** NMT 3.5%

**Table 2**

Name	Relative Retention Time	Relative Response Factor
Clarithromycin impurity I <sup>a</sup>	0.38	1.0
Clarithromycin impurity A <sup>b</sup> (clarithromycin F)	0.42	1.0
Clarithromycin impurity I <sup>c</sup>	0.63	1.0
Clarithromycin impurity L <sup>d</sup>	0.74	1.0
Clarithromycin impurity B <sup>e</sup>	0.79	1.0
Clarithromycin impurity M <sup>f</sup>	0.81	1.0
Clarithromycin impurity C <sup>g</sup>	0.89	1.0
Clarithromycin impurity D <sup>h</sup>	0.96	1.0
Clarithromycin	1.0	—
Clarithromycin impurity N <sup>i</sup>	1.15	1.0
Clarithromycin related compound A <sup>j</sup>	1.27	1.0
Clarithromycin impurity F <sup>k</sup>	1.33	1.0
Clarithromycin impurity P <sup>l</sup>	1.35	1.0
Clarithromycin impurity O <sup>m</sup>	1.38	1.0
Clarithromycin impurity K <sup>n</sup>	1.59	1.0
Clarithromycin impurity G <sup>o</sup>	1.72	3.7
Clarithromycin impurity H <sup>p</sup>	1.82	6.7

<sup>a</sup> 3-O-Decladienosyl-6-O-methylerythromycin A.

<sup>b</sup> 2-Deethyl-2-(hydroxymethyl)-6-O-methylerythromycin A.

<sup>c</sup> Erythromycin A (E)-9-oxime.

<sup>d</sup> 6-O-Methylerythromycin (Z)-9-oxime.

<sup>e</sup> 6-O-Methyl-15-norerythromycin A.

<sup>f</sup> 3''-N-Deethyl-6-O-methylerythromycin A (E)-9-oxime.

<sup>g</sup> 6-O-Methylerythromycin A (E)-9-oxime.

<sup>h</sup> 3''-N-Deethyl-6-O-methylerythromycin A.

<sup>i</sup> (10E)-10,11-Didehydro-11-deoxy-6-O-methylerythromycin A.

<sup>j</sup> 6,11-Di-O-methylerythromycin A.

<sup>k</sup> 6,12-Di-O-methylerythromycin A.

<sup>l</sup> 4',6-Di-O-methylerythromycin A.

<sup>m</sup> 6-O-Methylerythromycin A (Z)-9-(O-methyloxime).

<sup>n</sup> (1S,2R,5R,6S,7S,8R,9R,11Z)-2-Ethyl-6-hydroxy-9-methoxy-1,5,7,9,11,13-hexamethyl-8-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexapyranosyl]oxy]-3,15-dioxabicyclo[10.2.1]pentadeca-11,13-dien-4-one (3-O-decladienosyl-8,9:10,11-dianhydro-6-O-methylerythromycin A)-9,12-hemiketal.

<sup>o</sup> 6-O-Methylerythromycin A (E)-9-(O-methyloxime).

<sup>p</sup> 3''-N-Deethyl-3''-N-formyl-6-O-methylerythromycin A.

**SPECIFIC TESTS****• OPTICAL ROTATION (781S), Specific Rotation**

Sample solution: 10 mg/mL in methylene chloride

Acceptance criteria: −94° to −102° (at 20°)

**• CRYSTALLINITY (695):** Meets the requirements**• PH (791)**

Sample: 2-mg/mL suspension in methanol and water (1:19)

Acceptance criteria: 8.0–10.0

**• WATER DETERMINATION (921), Method I:** NMT 2.0%**ADDITIONAL REQUIREMENTS****• PACKAGING AND STORAGE:** Preserve in tight containers.**• USP REFERENCE STANDARDS (11)**

USP Clarithromycin RS

USP Clarithromycin Identity RS

This is a mixture of clarithromycin, clarithromycin impurity D (3''-N-demethyl-6-O-methylerythromycin A; C<sub>37</sub>H<sub>67</sub>NO<sub>13</sub> 733.9), and other impurities.



## Clarithromycin for Oral Suspension

### DEFINITION

Clarithromycin for Oral Suspension is a dry mixture of Clarithromycin, dispersing agents, diluents, preservatives, and flavorings. It contains NLT 90.0% and NMT 115.0% of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ), the labeled amount being 25 mg or 50 mg/mL when constituted as directed in the labeling.

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Buffer A:** 0.067 M monobasic potassium phosphate

**Buffer B:** 0.067 M dibasic potassium phosphate

**Mobile phase:** Methanol and *Buffer A* (60:40), adjusted with phosphoric acid to a pH of 3.5. Pass through a suitable filter.

**Standard stock solution:** Equivalent to 2.1 mg/mL of clarithromycin from USP Clarithromycin RS in methanol

**Standard solution:** 0.415 mg/mL of clarithromycin from *Standard stock solution* in *Mobile phase*

**Sample stock solution:** Constitute the Clarithromycin for Oral Suspension as directed in the labeling. Transfer an aliquot of the suspension, equivalent to 1–2 g of clarithromycin, with the aid of 330 mL of *Buffer B*, to a 1000-mL volumetric flask containing 50 mL of *Buffer B*. Shake by mechanical means for 30 min, and dilute with methanol to volume. Sonicate for about 30 min, and allow to cool. Dilute with methanol to volume, add a magnetic stirring bar, and stir for 60 min. Allow to settle, and use the clear supernatant.

**Sample solution:** Transfer an aliquot of the *Sample stock solution*, nominally equivalent to 20 mg of clarithromycin, to a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and pass through a suitable filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

#### Columns

**Guard (optional):** Packing L1

**Analytical:** 4.6-mm × 15-cm; packing L1

**Column temperature:** 50°

**Flow rate:** 1 mL/min

**Injection volume:** 50 µL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** 1.0–1.7

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) in the portion of the constituted Clarithromycin for Oral Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area response from the *Sample solution*

$r_S$  = peak area response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clarithromycin in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–115.0%

### PERFORMANCE TESTS

- UNIFORMITY OF DOSAGE UNITS (905)** (for powder packaged in single-unit containers): Meets the requirements
- DELIVERABLE VOLUME (698)** (for powder packaged in multiple-unit containers): Meets the requirements

### SPECIFIC TESTS

#### PH (791)

**Sample:** Use the suspension constituted as directed in the labeling.

**Acceptance criteria:** 4.0–5.4

#### LOSS ON DRYING (731)

**Sample:** 1 g

**Analysis:** Dry under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

**Acceptance criteria:** NMT 2.0%

### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in tight containers.
- USP REFERENCE STANDARDS (11)**  
USP Clarithromycin RS

## Clarithromycin Tablets

### DEFINITION

Clarithromycin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ).

### IDENTIFICATION

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Mobile phase:** Methanol and 0.067 M monobasic potassium phosphate (13:7). Adjust with phosphoric acid to a pH of 4.0, and pass through a suitable filter.

**System suitability stock solution:** 625 µg/mL of USP Clarithromycin Related Compound A RS in methanol

**System suitability solution:** 125 µg/mL of USP Clarithromycin RS from the *Standard stock solution* and 125 µg/mL of USP Clarithromycin Related Compound A RS from the *System suitability stock solution* in *Mobile phase*

**Standard stock solution:** 625 µg/mL of clarithromycin from USP Clarithromycin RS dissolved in methanol. Shake, and sonicate to facilitate dissolution.

**Standard solution:** 125 µg/mL of clarithromycin from *Standard stock solution* in *Mobile phase*. Pass through a suitable filter.

**Sample stock solution:** Nominally 4 mg/mL of clarithromycin from finely powdered Tablets in methanol. Shake by mechanical means for 30 min to disperse, and allow any insoluble matter to settle.

**Sample solution:** 120 µg/mL of clarithromycin from the *Sample stock solution* in *Mobile phase*. Pass through a filter of 0.5-µm or finer pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

[NOTE—A guard column containing packing L1 may be added.]



Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 20–50 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clarithromycin and clarithromycin related compound A are 0.75 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.0 between clarithromycin and clarithromycin related compound A, *System suitability solution*

**Tailing factor:** 0.9–1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of clarithromycin in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Buffer:** Prepare a solution containing 13.61 mg/mL of sodium acetate trihydrate in water. Prepare another solution by diluting 5.7 mL of glacial acetic acid with water to 1 L. Combine portions of the two solutions to obtain a pH of 5.0.

**Medium:** *Buffer*, 900 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Mobile phase, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Standard stock solution:** 625 µg/mL of clarithromycin from USP Clarithromycin RS dissolved in *Buffer*. Shake, and sonicate to facilitate dissolution.

**Standard solution:** 125 µg/mL of clarithromycin from the *Standard stock solution* in *Mobile phase*. Pass through a suitable filter.

**Sample solution:** Dilute with *Mobile phase* to yield a solution containing nominally 125 µg/mL of clarithromycin.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of the *Sample solution* (µg/mL)

**Tolerances:** NLT 80% (Q) of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

### IMPURITIES

#### • ORGANIC IMPURITIES

**Solution A:** 4.76 g/L of monobasic potassium phosphate adjusted with dilute phosphoric acid (1 in 10) or 4.5% (w/v) of potassium hydroxide to a pH of 4.4

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	75	25
32	40	60
34	40	60
36	75	25
42	75	25

**Diluent:** Acetonitrile and water (1:1)

**System suitability solution:** 1.5 mg/mL of USP Clarithromycin Identity RS in acetonitrile and water (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume.

**Standard stock solution:** 1.5 mg/mL of USP Clarithromycin RS in acetonitrile and water (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume.

**Standard solution 1:** 0.075 mg/mL of USP Clarithromycin RS from *Standard stock solution* in *Diluent*

**Standard solution 2:** 0.0075 mg/mL of USP Clarithromycin RS from *Standard solution 1* in *Diluent*

**Sample solution:** Nominally 1.5 mg/mL of clarithromycin from finely powdered Tablets in acetonitrile and water (1:1). Dissolve first in acetonitrile, using 50% of the final volume, and dilute with water to volume. Sonicate, and pass through a suitable filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 205 nm

**Column:** 4.6-mm × 10-cm; 3.5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.1 mL/min

**Injection volume:** 10 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution 1*

[NOTE—See *Table 2* for relative retention times. The typical retention time for clarithromycin is about 11 min.]

#### Suitability requirements

**Peak-to-valley ratio:** NLT 3.0 between clarithromycin and clarithromycin impurity D, *System suitability solution*. Calculate as follows:

$$\text{Result} = H_P/H_V$$

$H_P$  = height above the baseline of the clarithromycin impurity D peak

$H_V$  = height above the baseline of the lowest point of the curve separating the clarithromycin impurity D peak from the clarithromycin peak

**Tailing factor:** NMT 1.7, *Standard solution 1*

#### Analysis

**Samples:** *Standard solution 2* and *Sample solution*

Calculate the percentage of each impurity in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from *Standard solution 2*

$C_S$  = concentration of clarithromycin in *Standard solution 2* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** The reporting threshold is 0.1%. Disregard the peaks eluting before impurity I and after impurity H.



Any individual impurity: NMT 1.0%; NMT four impurities exceed 0.4%.

Total impurities: NMT 3.5%

Table 2

Name	Relative Retention Time	Relative Response Factor
Clarithromycin impurity Ia	0.38	1.0
Clarithromycin impurity Ab (clarithromycin F)	0.42	1.0
Clarithromycin impurity Jc	0.63	1.0
Clarithromycin impurity Ld	0.74	1.0
Clarithromycin impurity Be	0.79	1.0
Clarithromycin impurity Mi	0.81	1.0
Clarithromycin impurity Cg	0.89	1.0
Clarithromycin impurity Dh	0.96	1.0
Clarithromycin	1.0	—
Clarithromycin impurity Ni	1.15	1.0
Clarithromycin related compound Ai	1.27	1.0
Clarithromycin impurity Fh	1.33	1.0
Clarithromycin impurity Pl	1.35	1.0
Clarithromycin impurity Om	1.38	1.0
Clarithromycin impurity Kn	1.59	1.0
Clarithromycin impurity Go	1.72	3.7
Clarithromycin impurity Hp	1.82	6.7

a 3-O-Decladinosyl-6-O-methylerythromycin A.

b 2-Demethyl-2-(hydroxymethyl)-6-O-methylerythromycin A.

c Erythromycin A (E)-9-oxime.

d 6-O-Methylerythromycin (Z)-9-oxime.

e 6-O-Methyl-15-norerythromycin A.

f 3''-N-Demethyl-6-O-methylerythromycin A (E)-9-oxime.

g 6-O-Methylerythromycin A (E)-9-oxime.

h 3''-N-Demethyl-6-O-methylerythromycin A.

i (10E)-10,11-Didehydro-11-deoxy-6-O-methylerythromycin A.

j 6,11-Di-O-methylerythromycin A.

k 6,12-Di-O-methylerythromycin A.

l 4',6-Di-O-methylerythromycin A.

m 6-O-Methylerythromycin A (Z)-9-(O-methyloxime).

n (1S,2R,5R,6S,7S,8R,9R,11Z)-2-Ethyl-6-hydroxy-9-methoxy-1,5,7,9,11,13-hexamethyl-8-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexapyranosyl]oxy]-3,15-dioxabicyclo[10.2.1]pentadeca-11,13-dien-4-one (3-O-decladinosyl-8,9:10,11-dianhydro-6-O-methylerythromycin A)-9,12-hemiketal.

o 6-O-Methylerythromycin A (E)-9-(O-methyloxime).

p 3''-N-Demethyl-3''-N-formyl-6-O-methylerythromycin A.

## SPECIFIC TESTS

### • LOSS ON DRYING (731)

**Analysis:** Dry a portion of powdered Tablets under vacuum at a pressure not exceeding 5 mm of mercury at 110° for 3 h.

**Acceptance criteria:** NMT 6.0%

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS (11)**

USP Clarithromycin RS

USP Clarithromycin Identity RS

This is a mixture of clarithromycin, clarithromycin impurity D (3''-N-demethyl-6-O-methylerythromycin A; C<sub>37</sub>H<sub>67</sub>NO<sub>13</sub> 733.9), and other impurities.

USP Clarithromycin Related Compound A RS

6,11-Di-O-methylerythromycin A.

C<sub>39</sub>H<sub>71</sub>NO<sub>13</sub> 761.98

## Clarithromycin Extended-Release Tablets

### DEFINITION

Clarithromycin Extended-Release Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clarithromycin (C<sub>38</sub>H<sub>69</sub>NO<sub>13</sub>).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### • PROCEDURE

**Buffer A:** 0.067 M monobasic potassium phosphate  
**Mobile phase:** Methanol and *Buffer A* (13:7). Adjust with phosphoric acid to a pH of 4.0. Pass through a suitable filter.

**Standard stock solution:** 625 µg/mL of clarithromycin from USP Clarithromycin RS in methanol. Shake and sonicate, if necessary, to facilitate dissolution.

**Standard solution:** 125 µg/mL of clarithromycin in *Mobile phase* from *Standard stock solution*. Pass through a suitable filter.

**System suitability stock solution:** 625 µg/mL of USP Clarithromycin Related Compound A RS in methanol

**System suitability solution:** 125 µg/mL of USP Clarithromycin Related Compound A RS from *System suitability stock solution* and 125 µg/mL of clarithromycin from *Standard stock solution* in *Mobile phase*

**Sample stock solution:** Transfer nominally 2000 mg of clarithromycin from finely powdered Tablets to a 500-mL volumetric flask with the aid of methanol. Add about 350 mL of methanol, and shake by mechanical means for 30 min. Dilute with methanol to volume, and sonicate for 30 min. Cool to room temperature, and allow to stand for at least 16 h. Mix, allow any insoluble matter to settle, and use the supernatant.

**Sample solution:** Transfer 3.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass through a suitable filter.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Columns**

**Guard (optional):** Packing L1

**Analytical:** 4.6-mm × 15-cm; packing L1

**Column temperature:** 50°

**Flow rate:** 1 mL/min

**Injection volume:** 20–50 µL

### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clarithromycin and clarithromycin related compound A are about 0.75 and 1.0, respectively.]

### Suitability requirements

**Resolution:** NLT 2.0 between clarithromycin and clarithromycin related compound A, *System suitability solution*

**Tailing factor:** 0.9–1.5, *Standard solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clarithromycin (C<sub>38</sub>H<sub>69</sub>NO<sub>13</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$



- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of clarithromycin in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_U$  = nominal concentration of clarithromycin in the *Sample solution* ( $\mu\text{g/mL}$ )

Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

#### Test 1

**Buffer B:** Dissolve 816.5 g of monobasic potassium phosphate and 48 g of sodium hydroxide in about 4 L of water, mix, and dilute with water to 20 L. Adjust with either concentrated phosphoric acid or 1 N sodium hydroxide to a pH of  $6.0 \pm 0.05$ .

**Medium:** *Buffer B*; 900 mL

**Apparatus 2:** 75 rpm

**Times:** 30, 45, 60, and 120 min

**Standard solutions:** Prepare five solutions of USP Clarithromycin RS dissolved in acetonitrile and diluted with *Medium*, with known concentrations over a range of about 60–600  $\mu\text{g/mL}$ .

**Sample solution:** Use portions of the solution under test passed through a polyethylene filter of 35- $\mu\text{m}$  pore size.

**Chromatographic system:** Proceed as directed in the *Assay*, except the *Injection volume* is 50  $\mu\text{L}$ .

#### Analysis

**Samples:** *Standard solutions* and *Sample solution*

Perform a linear regression analysis to generate a standard curve using the peak area of each *Standard solution* versus its concentration. Determine the percentage of the labeled amount of clarithromycin ( $\text{C}_{38}\text{H}_{69}\text{NO}_{13}$ ) dissolved at each specified time interval, using the peak area of each *Sample solution* and the linear regression statistics for the *Standard solutions*.

**Tolerances:** The percentages of the labeled amounts of clarithromycin ( $\text{C}_{38}\text{H}_{69}\text{NO}_{13}$ ) dissolved at the times specified conform to *Table 1*.

Table 1

Level	Time (min)	Amount Dissolved, Individual Limits (%)	Amount Dissolved, Average Limits (%)
L1	30	NMT 65	—
	45	55–85	—
	60	NLT 75	—
	120	NLT 85	—
L2	30	NMT 75	NMT 65
	45	45–95	55–85
	60	NLT 65	NLT 75
	120	NLT 75	NLT 85

Table 1 (Continued)

Level	Time (min)	Amount Dissolved, Individual Limits (%)	Amount Dissolved, Average Limits (%)
L3	30	NMT 2 Tablets release more than 75%, and no individual Tablet releases more than 85%	NMT 65
	45	NMT 2 Tablets are outside the range of 45%–95%, and no individual Tablet is outside the range of 35%–105%	55–85
	60	NMT 2 Tablets release less than 65%, and no individual Tablet releases less than 55%	NLT 75
	120	NMT 2 Tablets release less than 75%, and no individual Tablet releases less than 65%	NLT 85

#### Test 2

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Buffer C:** 0.05 M phosphate buffer with a pH of 6.8, containing 0.5% of sodium lauryl sulfate

**Medium:** *Buffer C*; 900 mL, degassed by sonication and vacuum

**Apparatus 1:** 100 rpm

**Times:** 2, 12, and 24 h

**Buffer D:** 9.2 g/L of monobasic sodium phosphate monohydrate in water, adjusted with phosphoric acid to a pH of 2.5 prior to final dilution

**Mobile phase:** Methanol and *Buffer D* (65:35)

**Standard solution:** 0.56 mg/mL of USP Clarithromycin RS in a solution of methanol and *Medium* (1 in 10). Dissolve first in methanol using 10% of the final volume, and dilute with *Medium* to volume.

**Sample solution:** Centrifuge the solution under test at 2500 rpm for 10 min.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 5 μL

**System suitability**Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at each time point ( $Q_t$ ):

$$Q_2 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_{12} = [Q_2 \times (V_S/V)] + [(r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100]$$

$$Q_{24} = [Q_2 \times (V_S/V)] + [Q_{12} \times V_S/(V - 2V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 2V_S) \times 100]$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of clarithromycin in the *Standard solution* (mg/mL) $V$  = volume of *Medium*, 900 mL $V_S$  = volume of the sample withdrawn at each time point (mL) $L$  = label claim (mg/Tablet)**Tolerances:** The percentages of the labeled amounts of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* (711).**Table 2**

Time (h)	Amount Dissolved (%)
2	NMT 20
12	45–70
24	NLT 80

**Test 3**If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.**Buffer E:** Dissolve 3.59 g of sodium acetate trihydrate and 11.0 mL of 2 N acetic acid in 1000 mL of water. Adjust with 2 N acetic acid to a pH of 4.75.**Medium:** *Buffer E*; 1000 mL**Apparatus 1:** 10 mesh; 50 rpm**Times:** 1, 2, 4, 8, and 12 h**Buffer F:** 9.12 g/L of monobasic potassium phosphate in water**Mobile phase:** Methanol and *Buffer F* (65:35). Adjust with phosphoric acid to a pH of 4.0.**Standard stock solution:** 625 μg/mL of clarithromycin from USP Clarithromycin RS in methanol. Shake and sonicate, if necessary, to dissolve.**Standard solution:** 125 μg/mL of clarithromycin from *Standard stock solution* in *Mobile phase***System suitability stock solution:** 625 μg/mL of USP Clarithromycin Related Compound A RS in methanol**System suitability solution:** 125 μg/mL of clarithromycin related compound A from *System suitability stock solution* and 125 μg/mL of clarithromycin from *Standard stock solution* in *Mobile phase***Sample solution:** Withdraw 10 mL of the solution under test from each vessel and replace with 10 mL of *Medium*. Transfer 3 mL of the withdrawn solution to a 25-mL volumetric flask, and dilute with *Mobile phase* to volume. Pass through a filter of 0.45-μm pore size.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 50 μL

**System suitability**Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clarithromycin and clarithromycin related compound A are about 0.75 and 1.0, respectively.]

**Suitability requirements****Resolution:** NLT 2.0 between clarithromycin and clarithromycin related compound A, *System suitability solution***Tailing factor:** 0.9–2, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at each time point ( $Q_t$ ):

$$Q_1 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_2 = [Q_1 \times (V_S/V)] + [(r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100]$$

$$Q_4 = [Q_1 \times (V_S/V)] + [Q_2 \times V_S/(V - 2V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 2V_S) \times 100]$$

$$Q_8 = [Q_1 \times (V_S/V)] + [Q_2 \times V_S/(V - 2V_S)] + [Q_4 \times V_S/(V - 3V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 3V_S) \times 100]$$

$$Q_{12} = [Q_1 \times (V_S/V)] + [Q_2 \times V_S/(V - 2V_S)] + [Q_4 \times V_S/(V - 3V_S)] + [Q_8 \times V_S/(V - 4V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 4V_S) \times 100]$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of clarithromycin in the *Standard solution* (mg/mL) $V$  = volume of *Medium*, 1000 mL $V_S$  = volume of the sample withdrawn at each time point (mL) $L$  = label claim (mg/Tablet)**Tolerances:** The percentages of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at the times specified conform to *Acceptance Table 2* in *Dissolution* (711).**Table 3**

Time (h)	Amount Dissolved (%)
1	NMT 15
2	10–30
4	35–55
8	NLT 80
12	NLT 90



**Test 4**

If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

**Buffer G:** 6.8 g/L of potassium dihydrogen phosphate and 0.18 g/L of sodium hydroxide in water. Adjust with dilute sodium hydroxide or phosphoric acid to a pH of  $6.0 \pm 0.1$ .

**Medium:** Buffer G; 900 mL

**Apparatus 2:** 50 rpm

**Times:** 2, 4, 8, and 12 h

**Buffer H:** 6.8 g/L of potassium dihydrogen phosphate in water. Adjust with dilute sodium hydroxide or phosphoric acid to a pH of  $4.5 \pm 0.1$ .

**Mobile phase:** Methanol and Buffer H (64:36)

**Standard solution:** 0.4 mg/mL of USP Clarithromycin RS in methanol and Medium (4:96). Dissolve first in Medium, using 60% of the final volume. Sonicate about 10 min until dissolved. Add methanol, using 4% of the final volume. Dilute with Medium to volume.

**Sample solution:** Use the solution under test, passed through a suitable filter of 0.45- $\mu$ m pore size.

**Chromatographic system**

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 203 nm

**Column:** 4.0-mm  $\times$  12.5-cm; 5- $\mu$ m packing L7

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

**System suitability**

**Sample:** Standard solution

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** Standard solution and Sample solution

Determine the concentration, in mg/mL, of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) in the Sample solution at each time point:

$$\text{Result} = (r_U/r_S) \times C_S$$

- $r_U$  = peak response from the Sample solution  
 $r_S$  = peak response from the Standard solution  
 $C_S$  = concentration of the Standard solution (mg/mL)

Calculate the percentage of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at each time point ( $Q_t$ ):

$$Q_2 = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$$Q_4 = [Q_2 \times (V_S/V)] + [(r_U/r_S) \times (C_S/L) \times (V - V_S) \times 100]$$

$$Q_8 = [Q_2 \times (V_S/V)] + [Q_4 \times V_S/(V - 2V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 2V_S) \times 100]$$

$$Q_{12} = [Q_2 \times (V_S/V)] + [Q_4 \times V_S/(V - 2V_S)] + [Q_8 \times V_S/(V - 3V_S)] + [(r_U/r_S) \times (C_S/L) \times (V - 3V_S) \times 100]$$

- $r_U$  = peak response from the Sample solution  
 $r_S$  = peak response from the Standard solution  
 $C_S$  = concentration of clarithromycin in the Standard solution (mg/mL)  
 $V$  = volume of Medium, 900 mL  
 $V_S$  = volume of the sample withdrawn at each time point (mL)  
 $L$  = label claim (mg/Tablet)

**Tolerances:** The percentages of the labeled amount of clarithromycin ( $C_{38}H_{69}NO_{13}$ ) dissolved at the times specified conform to Acceptance Table 2 in Dissolution <711>.

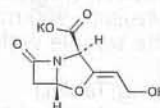
**Table 4**

Time (h)	Amount Dissolved (%)
2	NMT 25
4	20–40
8	45–75
12	NLT 80

- **UNIFORMITY OF DOSAGE UNITS <905>:** Meet the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at 25°, excursions permitted between 15° and 30°.
- **LABELING:** When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.
- **USP REFERENCE STANDARDS <11>**  
 USP Clarithromycin RS  
 USP Clarithromycin Related Compound A RS  
 6,11-Di-O-methylerythromycin A  
 $C_{39}H_{71}NO_{13}$  762.00

**Clavulanate Potassium**

$C_8H_8KNO_5$  237.25  
 4-Oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 3-(2-hydroxyethylidene)-7-oxo-, monopotassium salt, 2R-(2 $\alpha$ ,3Z,5 $\alpha$ )-;  
 Potassium (Z)-(2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate [61177-45-5].

**DEFINITION**

Clavulanate Potassium contains the equivalent of NLT 75.5% and NMT 92.0% of clavulanic acid ( $C_8H_9NO_5$ ), calculated on the anhydrous basis.

**IDENTIFICATION**

- **A.** The retention time of the major peak for clavulanic acid in the Sample solution corresponds to that in the Standard solution, as obtained in the Assay.
- **B. IDENTIFICATION TESTS—GENERAL, Potassium <191>:** Meets the requirements

**ASSAY****PROCEDURE**

**Solution A:** 7.8 mg/mL of monobasic sodium phosphate in water. Adjust with phosphoric acid or 10 N sodium hydroxide to a pH of  $4.4 \pm 0.1$  before final dilution.

**Mobile phase:** Methanol and Solution A (1:19)

**Standard solution:** 0.25 mg/mL of USP Clavulanate Lithium RS in water

**System suitability solution:** 0.5 mg/mL of amoxicillin dissolved in Standard solution

**Sample solution:** 0.25 mg/mL of Clavulanate Potassium in water



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 220 nm**Column:** 4-mm × 30-cm; 3- to 10-μm packing L1**Flow rate:** 2 mL/min**Injection size:** 20 μL**System suitability****Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clavulanic acid and amoxicillin are about 0.5 and 1.0, respectively.]

**Suitability requirements****Resolution:** NLT 3.5 between the amoxicillin and clavulanic acid peaks, *System suitability solution***Column efficiency:** NLT 550 theoretical plates, *Standard solution***Tailing factor:** NMT 1.5, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of C<sub>8</sub>H<sub>9</sub>NO<sub>5</sub> in each mg of Clavulanate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Clavulanate Lithium RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of Clavulanate Potassium in the *Sample solution* (mg/mL) $P$  = designated potency of USP Clavulanate Lithium RS, in μg/mg of clavulanic acid $F$  = unit conversion factor, 0.001 mg/μg**Acceptance criteria:** 75.5%–92.0% on the anhydrous basis**IMPURITIES****Organic Impurities****• PROCEDURE 1****Solution A:** 0.05 M monobasic sodium phosphate. Adjust with phosphoric acid to a pH of 4.0 ± 0.1.**Solution B:** Methanol and *Solution A* (1:1)**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
15	50	50
18	50	50
24	100	0

**Standard solution:** 0.1 mg/mL of USP Clavulanate Lithium RS in *Solution A***Sample solution:** 10.0 mg/mL of Clavulanate Potassium in *Solution A***System suitability solution:** 0.1 mg/mL each of USP Clavulanate Lithium RS and amoxicillin in *Solution A***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 230 nm**Column:** 4.6-mm × 10-cm; 5-μm packing L1**Temperature:** 40°**Flow rate:** 1 mL/min[NOTE—The system is equilibrated for 15 min with 100% *Solution A*.]**Injection size:** 20 μL**System suitability****Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clavulanic acid and amoxicillin are about 1.0 and 2.5, respectively.]

**Suitability requirements****Resolution:** NLT 13 between the clavulanic acid peak and the amoxicillin peak, *System suitability solution***Column efficiency:** NLT 2000 theoretical plates from the clavulanic acid peak, *System suitability solution***Tailing factor:** NMT 2.0 for the clavulanic acid peak, *System suitability solution***Relative standard deviation:** NMT 2%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage, in terms of clavulanate potassium equivalent, of each impurity in the Clavulanate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

 $r_U$  = peak response of an individual impurity peak from the *Sample solution* $r_S$  = peak response of clavulanic acid from the *Standard solution* $C_S$  = concentration of the *Standard solution* (mg/mL) $C_U$  = nominal concentration of Clavulanate Potassium from the *Sample solution* (mg/mL) $M_{r1}$  = molecular weight of clavulanate potassium, 237.3 $M_{r2}$  = molecular weight of clavulanate lithium, 205.1**Acceptance criteria****Total impurities:** NMT 2%**• PROCEDURE 2: LIMIT OF CLAVAM-2-CARBOXYLATE POTASSIUM****Mobile phase:** 0.1 M monobasic sodium phosphate.

Adjust with phosphoric acid to a pH of 4.0 ± 0.1.

**Standard solution:** 5 μg/mL of USP Clavam-2-Carboxylate Potassium RS in water**Sample solution:** 10 mg/mL of Clavulanate Potassium in water**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 210 nm**Column:** 4-mm × 30-cm; 3- to 10-μm packing L1**Flow rate:** 0.5 mL/min**Injection size:** 20 μL**System suitability****Sample:** *Standard solution*

[NOTE—The relative retention times for clavam-2-carboxylic acid and clavulanic acid are about 0.7 and 1.0, respectively.]

**Suitability requirements****Column efficiency:** NLT 4000 theoretical plates**Tailing factor:** NMT 1.5**Relative standard deviation:** NMT 5%**Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clavam-2-carboxylate potassium in the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times F \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of the *Standard solution* (μg/mL) $C_U$  = nominal concentration of Clavulanate Potassium in the *Sample solution* (mg/mL) $F$  = unit conversion factor, 0.001 mg/μg



Acceptance criteria: NMT 0.01%

• **PROCEDURE 3: LIMIT OF ALIPHATIC AMINES**

**Internal standard solution:** 50 µL of 3-methyl-2-pentanone in water to 100 mL

**Standard solution:** Dissolve 80.0 mg of each of the following amines in 2 N hydrochloric acid: 1,1-dimethylethylamine, diethylamine, tetramethylethylenediamine, 1,1,3,3-tetramethylbutylamine, and *N,N'*-diisopropylethylenediamine. Dilute with 2 N hydrochloric acid to 200.0 mL. Transfer 5.0 mL of this solution to a centrifuge tube. Add 5.0 mL of *Internal standard solution*, 10.0 mL of 2 N sodium hydroxide, 5.0 mL of isopropyl alcohol, and 5 g of sodium chloride. Shake for 1 min, and centrifuge to separate the layers. Use the upper layer.

**Sample solution:** Transfer 1.0 g of Clavulanate Potassium to a centrifuge tube, add 5.0 mL of *Internal standard solution*, 5.0 mL of 2 N sodium hydroxide, 10.0 mL of water, 5.0 mL of isopropyl alcohol, and 5 g of sodium chloride. Shake for 1 min, and centrifuge to separate the layers. Use the upper layer.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm × 50-m capillary fused silica column that contains a 5-µm film coating of stationary phase G41

**Temperature**

**Injector:** 200°

**Detector:** 250°

**Column:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
35	—	35	7
35	30	150	15

**Carrier gas:** Helium

**Flow rate:** 8 mL/min

**Split ratio:** 1:10

**Injection size:** 1 µL

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

[NOTE—See the table below for relative retention times.]

Name	Relative Retention Time
1,1-Dimethylethylamine	0.55
Diethylamine	0.76
3-Methyl-2-pentanone (internal standard)	1.0
Tetramethylethylenediamine	1.07
1,1,3,3-Tetramethylbutylamine	1.13
<i>N,N'</i> -Diisopropylethylenediamine	1.33
Bis(2-methylamino)ethyl ether <sup>a</sup>	1.57

<sup>a</sup> The relative retention time for this compound is provided for information only; bis(2-methylamino)ethyl ether is not a component of the *Standard solution*.

Calculate the percentage of each impurity in the Clavulanate Potassium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response for an individual impurity from the *Sample solution*

$r_S$  = peak response for the relevant analyte from the *Standard solution*

$C_S$  = concentration of the relevant analyte in the *Standard solution*

$C_U$  = nominal concentration of Clavulanate Potassium in the *Sample solution*

Calculate the percentage of any individual impurity for which no relevant reference compound is provided in the *Standard solution* by the same formula, except for  $r_S$  use the peak response corresponding to the 1,1-dimethylethylamine peak.

**Acceptance criteria**

**Total of all aliphatic amines:** NMT 0.2%

• **PROCEDURE 4: LIMIT OF 2-ETHYLHEXANOIC ACID**

**Internal standard solution:** 1 mg/mL of 3-cyclohexylpropionic acid in cyclohexane

**Standard solution:** 1.5 mg/mL of 2-ethylhexanoic acid in *Internal standard solution*. Transfer 1.0 mL of this solution to a centrifuge tube, and add 4.0 mL of 4 N hydrochloric acid. Shake for 1 min, and allow the phases to separate, centrifuging if necessary. Withdraw the lower phase, and reserve the upper phase. To the lower phase add 1.0 mL of *Internal standard solution*, and shake for 1 min. Allow the phases to separate, centrifuging if necessary. Withdraw the upper phase, and combine with the reserved upper layer.

**Sample solution:** Transfer 300 mg of Clavulanate Potassium to a centrifuge tube. Add 4.0 mL of 4 N hydrochloric acid, and shake with two successive 1.0-mL portions of the *Internal standard solution*. Allow the phases to separate, centrifuging if necessary. Use the combined upper phases.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.53-mm × 25-m capillary fused silica; 1-µm film coating of stationary phase G35

**Temperature**

**Injector temperature:** 200°

**Detector temperature:** 300°

**Column temperature:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
40	—	40	2
40	30	200	3

**Carrier gas:** Hydrogen

**Flow rate:** 100 cm/s

**Injection size:** 1 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between the 2-ethylhexanoic acid peak and the 3-cyclohexylpropionic acid peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 2-ethylhexanoic acid in the Clavulanate Potassium taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak area response ratio of 2-ethylhexanoic acid to 3-cyclohexylpropionic acid from the *Sample solution*

$R_S$  = peak area response ratio of 2-ethylhexanoic acid to 3-cyclohexylpropionic acid from the *Standard solution*

$C_S$  = concentration of 2-ethylhexanoic acid in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Clavulanate Potassium in the *Sample solution* (mg/mL)



Acceptance criteria: NMT 0.8%

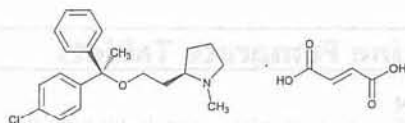
### SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Clavulanate Potassium is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.03 USP Endotoxin Unit/mg.
- **STERILITY TESTS (71):** Where the label states that Clavulanate Potassium is sterile, it meets the requirements when tested as directed under *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **PH (791):** 5.5–8.0, in a 10 mg/mL solution
- **WATER DETERMINATION, Method I (921):** NMT 1.5%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS (11)**
  - USP Clavam-2-Carboxylate Potassium RS
  - USP Clavulanate Lithium RS
  - USP Endotoxin RS

## Clemastine Fumarate



$C_{21}H_{26}ClNO \cdot C_4H_4O_4$  459.96  
 Pyrrolidine, 2-[2-[1-(4-chlorophenyl)-1-phenylethoxy]ethyl]-1-methyl-, [R-(R\*,R\*)]-, (E)-2-butenedioate (1:1);  
 (+)-(2R)-2-[2-[[[R]-p-chloro-α-methyl-α-phenylbenzyl]-oxy]ethyl]-1-methylpyrrolidine fumarate (1:1) [14976-57-9].

### DEFINITION

Clemastine Fumarate contains NLT 98.0% and NMT 102.0% of clemastine fumarate ( $C_{21}H_{26}ClNO \cdot C_4H_4O_4$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention times of the fumarate and clemastine peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Buffer:** 4.1 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (35:35:30)

**Standard solution:** 0.28 mg/mL of USP Clemastine Fumarate RS in *Mobile phase*

**Sample solution:** 0.28 mg/mL of Clemastine Fumarate in *Mobile phase*

#### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 5-cm; 1.8-μm packing L7

**Flow rate:** 1.2 mL/min

**Injection volume:** 5 μL

**Run time:** 3 min

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 1.8 for clemastine

**Relative standard deviation:** NMT 0.73% for clemastine

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clemastine fumarate ( $C_{21}H_{26}ClNO \cdot C_4H_4O_4$ ) in the portion of Clemastine Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clemastine from the *Sample solution*

$r_S$  = peak response of clemastine from the *Standard solution*

$C_S$  = concentration of USP Clemastine Fumarate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clemastine Fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

#### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm (Official 1-

Jan-2018)

#### ORGANIC IMPURITIES

**Buffer:** 4.1 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.

**Solution A:** Methanol, acetonitrile, and *Buffer* (35:35:30)

**Solution B:** Methanol, acetonitrile, and *Buffer* (40:37.5:22.5)

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
3.1	0	100
18	0	100
18.1	100	0
25	100	0

**Standard stock solution 1:** 0.14 mg/mL of USP

Clemastine Fumarate RS in *Solution A*. Sonication may be needed to aid dissolution.

**Standard stock solution 2:** 0.14 mg/mL of USP

4-Chlorobenzophenone RS in methanol. Sonication may be needed to aid dissolution.

**Sensitivity solution:** 0.14 μg/mL of USP Clemastine

Fumarate RS in *Solution A* from *Standard stock solution 1*

**Standard solution:** 0.28 μg/mL of USP Clemastine Fumarate RS and 0.36 μg/mL of USP

4-Chlorobenzophenone RS in *Solution A* from *Standard stock solution 1* and *Standard stock solution 2*, respectively

**Sample solution:** 0.28 mg/mL of Clemastine Fumarate in *Solution A*



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 5-cm; 1.8-μm packing L7

Flow rate: 1.2 mL/min

Injection volume: 50 μL

**System suitability****Samples:** *Sensitivity solution* and *Standard solution*  
**Suitability requirements****Resolution:** NLT 1.5 between clemastine and 4-chlorobenzophenone, *Standard solution***Relative standard deviation:** NMT 2.0% for clemastine, *Standard solution***Signal-to-noise ratio:** NLT 50 for clemastine, *Sensitivity solution***Analysis****Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 4-chlorobenzophenone in the portion of Clemastine Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of 4-chlorobenzophenone from the *Sample solution* $r_S$  = peak response of 4-chlorobenzophenone from the *Standard solution* $C_S$  = concentration of USP 4-Chlorobenzophenone RS in the *Standard solution* (μg/mL) $C_U$  = concentration of Clemastine Fumarate in the *Sample solution* (μg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Clemastine Fumarate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of each individual unspecified impurity from the *Sample solution* $r_S$  = peak response of clemastine from the *Standard solution* $C_S$  = concentration of USP Clemastine Fumarate RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Clemastine Fumarate in the *Sample solution* (mg/mL)**Acceptance criteria:** See *Table 2*. Disregard peaks having areas less than 0.05% of clemastine.**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fumaric acid <sup>a</sup>	0.5	—
Clemastine	1.0	—
4-Chlorobenzophenone	1.7	0.15

<sup>a</sup> Salt counter ion is included in this table for identification purposes only.**Table 2 (Continued)**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual unspecified impurity	—	0.10
Total impurities	—	1.0

<sup>a</sup> Salt counter ion is included in this table for identification purposes only.**SPECIFIC TESTS****OPTICAL ROTATION, Specific Rotation (781S)**

Sample solution: 10 mg/mL in methanol

Acceptance criteria: +15.0° to +18.0° (*T* = 20°)**pH (791)**

Sample solution: 100 mg/mL suspension

Acceptance criteria: 3.2–4.2

**LOSS ON DRYING (731)**

Analysis: Dry at 105° to constant weight.

Acceptance criteria: NMT 0.5%

**ADDITIONAL REQUIREMENTS****PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at a temperature not exceeding 25°.**USP REFERENCE STANDARDS (11)**

USP 4-Chlorobenzophenone RS

4-Chlorobenzophenone.

C<sub>13</sub>H<sub>9</sub>ClO 216.66

USP Clemastine Fumarate RS

**Clemastine Fumarate Tablets****DEFINITION**Clemastine Fumarate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clemastine fumarate (C<sub>21</sub>H<sub>26</sub>ClNO · C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>).**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- B.** The UV spectrum of the clemastine peak of the *Diluted sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.

**ASSAY****PROCEDURE****Solution A:** 9.47 g/L of anhydrous dibasic sodium phosphate in water**Solution B:** 9.08 g/L of monobasic potassium phosphate in water**Solution C:** *Solution A* and *Solution B* (612:388)**Buffer:** *Solution C* and water (25:75)**Mobile phase:** Methanol and *Buffer* (83:17)**Diluent:** Methanol and water (50:50)**Standard solution:** 0.14 mg/mL of USP Clemastine Fumarate RS in *Diluent***Sample solution:** Transfer a quantity of NLT 20 finely powdered Tablets, equivalent to 14 mg of clemastine fumarate, to a 200-mL conical flask. Pipet 100 mL of *Diluent* into the flask, shake for 30 min, centrifuge, and filter the supernatant.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)



Mode: LC  
 Detector: UV 220 nm  
 Column: 4.6-mm × 25-cm; 10-μm packing L7  
 Flow rate: 4 mL/min  
 Injection volume: 100 μL

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

Relative standard deviation: NMT 1.5%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clemastine fumarate ( $C_{21}H_{26}ClNO \cdot C_4H_4O_4$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Clemastine Fumarate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clemastine fumarate in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

Buffer: Dissolve 20.0 g of citric acid monohydrate in 1000 mL of water, add 22.0 mL of sodium hydroxide solution (3 in 10) and 8.8 mL of hydrochloric acid, and dilute with water to 2000 mL. Adjust, if necessary, with sodium hydroxide solution (1 in 2) to a pH of 4.0.

Medium: *Buffer*; 500 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: USP Clemastine Fumarate RS in *Medium* with a similar concentration to the *Sample solution*

Sample solution: Centrifuge 60 mL of the solution under test for 20 min at 4000 rpm.

#### Instrumental conditions

Mode: UV

Analytical wavelength: About 420 nm

Blank: *Medium*

#### Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*  
 Transfer 50.0 mL of the *Samples* to individual 125-mL separatory funnels, and treat each of the solutions as follows. Add 10 mL of methyl orange solution (2 in 10,000), mix, add 20.0 mL of chloroform, shake simultaneously by mechanical means for 10 min, remove the chloroform layer, and centrifuge the chloroform layer for 10 min at 4000 rpm. Use the *Blank* to set the instrument.

Calculate the percentage of the labeled amount of clemastine fumarate ( $C_{21}H_{26}ClNO \cdot C_4H_4O_4$ ) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of clemastine fumarate ( $C_{21}H_{26}ClNO \cdot C_4H_4O_4$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

### IMPURITIES

#### • ORGANIC IMPURITIES

Buffer: 4.1 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 4.0.

Solution A: Methanol, acetonitrile, and *Buffer* (35:35:30)

Solution B: Methanol, acetonitrile, and *Buffer* (40:37.5:22.5)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3	100	0
3.1	0	100
18	0	100
18.1	100	0
25	100	0

Standard stock solution 1: 0.14 mg/mL of USP Clemastine Fumarate RS in *Solution A*. Sonicate for NLT 5 min or until dissolved.

Standard stock solution 2: 0.14 mg/mL of USP 4-Chlorobenzophenone RS in methanol. Sonicate for NLT 5 min or until dissolved.

System suitability solution: 2.8 μg/mL each of USP Clemastine Fumarate RS and USP 4-Chlorobenzophenone RS in *Solution A* from *Standard stock solution 1* and *Standard stock solution 2*

Sensitivity solution: 0.14 μg/mL of USP Clemastine Fumarate RS in *Solution A* from *Standard stock solution 1*

Standard solution: 2.8 μg/mL of USP Clemastine Fumarate RS in *Solution A* from *Standard stock solution 1*

Sample solution: Nominally 0.28 mg/mL of clemastine fumarate from Tablets in *Solution A* prepared as follows. Transfer a quantity of NLT 20 finely powdered Tablets, equivalent to 14 mg of clemastine fumarate, to a 50-mL volumetric flask. Add 25 mL of *Solution A*, shake the flask for NLT 30 min, and sonicate for NLT 15 min. Dilute with *Solution A* to volume. Pass an aliquot through a suitable filter of 0.45-μm pore size, discarding the first 3 mL of filtrate.

Diluted sample solution: Nominally 2.8 μg/mL of clemastine fumarate in *Solution A* from *Sample solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm. For *Identification test B*, use a diode array detector in the range of 200–300 nm.

Column: 4.6-mm × 5-cm; 1.8-μm packing L7

Flow rate: 1.2 mL/min

Injection volume: 50 μL

#### System suitability

Samples: *System suitability solution*, *Sensitivity solution*, and *Standard solution*

[NOTE—The relative retention times for clemastine and 4-chlorobenzophenone are 1.0 and 1.7, respectively.]

#### Suitability requirements

Resolution: NLT 1.5 between clemastine and 4-chlorobenzophenone, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

#### Analysis

Samples: *Standard solution*, *Diluted sample solution*, and *Sample solution*

[NOTE—The *Diluted sample solution* is used for *Identification test B*.]

Calculate the percentage of any individual unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each unspecified degradation product from the *Sample solution*

$r_S$  = peak response of clemastine from the *Standard solution*

$C_S$  = concentration of USP Clemastine Fumarate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clemastine fumarate in the *Sample solution* (mg/mL)



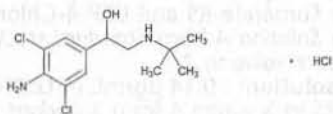
## Acceptance criteria

Any unspecified degradation product: NMT 0.5%  
Total impurities: NMT 2.0%

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP 4-Chlorobenzophenone RS  
4-Chlorobenzophenone.  
 $C_{13}H_9ClO$  216.66  
USP Clemastine Fumarate RS

## Clenbuterol Hydrochloride



$C_{12}H_{18}Cl_2N_2O \cdot HCl$  313.65  
Ethanol, 1-(4-amino-3,5-dichlorophenyl)-2-(tert-butylamino), hydrochloride;  
4-Amino- $\alpha$ -[(tert-butylamino)methyl]-3,5-dichlorobenzyl alcohol, hydrochloride [21898-19-1].

## DEFINITION

Clenbuterol Hydrochloride contains NLT 98.0% and NMT 102.0% of  $C_{12}H_{18}Cl_2N_2O \cdot HCl$ , calculated on the anhydrous basis.

## IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**  
[NOTE—Alternatively, *Infrared Absorption* (197A) may be used.]
- **B. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements

## ASSAY

## • PROCEDURE

**Sample solution:** Dissolve 0.25 g in 50 mL of alcohol, and add 5.0 mL of 0.01 N hydrochloric acid.

**Analysis:** Titrate with 0.1 N sodium hydroxide VS, determining the endpoint potentiometrically. Read the volume added between the two points of inflection. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N sodium hydroxide is equivalent to 31.37 mg of  $C_{12}H_{18}Cl_2N_2O \cdot HCl$ .

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

## IMPURITIES

## Inorganic Impurities

- **RESIDUE ON IGNITION (281):** NMT 0.1%, from 1–2 g

## Delete the following:

- **HEAVY METALS, Method II (231):** NMT 10 ppm (Official 1, Jan-2018)

## Organic Impurities

## • PROCEDURE

**Buffer:** Dissolve 3.0 g of sodium 1-decanesulfonate and 5.0 g of monobasic potassium phosphate in 900 mL of water, adjust with dilute phosphoric acid (1 in 10) to a pH of 3.0, and dilute with water to 1000 mL.

**Mobile phase:** Acetonitrile, methanol, and Buffer (2:2:6)

**System suitability solution:** 0.2 mg/mL each of USP Clenbuterol Related Compound B RS and Clenbuterol Hydrochloride in *Mobile phase*

**Sample solution 1:** 2.0 mg/mL of Clenbuterol Hydrochloride in *Mobile phase*

**Sample solution 2:** 2.0  $\mu$ g/mL of Clenbuterol Hydrochloride in *Mobile phase*, from *Sample solution 1*

## Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.0-mm  $\times$  12.5-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 0.5 mL/min

**Injection size:** 5  $\mu$ L

## System suitability

**Sample:** *System suitability solution*

## Suitability requirements

**Resolution:** NLT 4.0 between clenbuterol related compound B and clenbuterol

**Relative standard deviation:** NMT 2.0% for the clenbuterol peak

## Analysis

**Samples:** *Sample solution 1* and *Sample solution 2*

Calculate the percentage of impurities in the portion of  $C_{12}H_{18}Cl_2N_2O \cdot HCl$  taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from *Sample solution 1*

$r_S$  = peak response of clenbuterol from *Sample solution 2*

$C_S$  = concentration of Clenbuterol Hydrochloride in *Sample solution 2* (mg/mL)

$C_U$  = concentration of Clenbuterol Hydrochloride in *Sample solution 1* (mg/mL)

## Acceptance criteria

**Individual impurities:** 0.1%

**Total impurities:** NMT 0.2%

[NOTE—The reporting level for impurities is 0.05%.]

## SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation (781S)**

**Sample:** 30 mg/mL in water, filter as necessary

**Acceptance criteria:**  $-10^\circ$  to  $+10^\circ$  at 20°

- **pH (791):** 5.0–7.0

**Sample:** 50 mg/mL in carbon dioxide-free water

- **WATER DETERMINATION, Method I (921):** NMT 1.0%

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, protected from light. Store at room temperature.

- **LABELING:** Label it to indicate that it is for veterinary use only.

- **USP REFERENCE STANDARDS (11)**

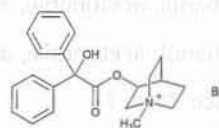
USP Clenbuterol Hydrochloride RS

USP Clenbuterol Related Compound B RS

1-(4-Amino-3,5-dichlorophenyl)-2-tert-butyl-aminoethanone hydrochloride.

$C_{12}H_{16}Cl_2N_2O \cdot HCl$  311.64

## Clidinium Bromide



$C_{22}H_{26}BrNO_3$

1-Azoniabicyclo[2.2.2]octane, 3-[(hydroxydiphenylacetyl)oxy]-1-methyl-, bromide, ( $\pm$ );

432.35



(±)-3-Hydroxy-1-methylquinuclidinium bromide benzilate [3485-62-9].

# DEFINITION

Clidinium Bromide contains NLT 99.0% and NMT 100.5% of  $C_{22}H_{26}BrNO_3$ , calculated on the dried basis.

# IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.
- **C. BROMIDE**  
*Sample solution:* 50 mg/mL  
*Analysis:* To 2 mL of the *Sample solution* add a few drops of 2 N nitric acid and 1 mL of silver nitrate TS.  
*Acceptance criteria:* A yellowish white precipitate is formed.

# ASSAY

- **PROCEDURE**  
*Sample:* 1.2 g  
*Analysis:* Dissolve the *Sample* in 80 mL of glacial acetic acid, warming if necessary to effect solution. Cool, and add 15 mL of mercuric acetate TS. Titrate with 0.1 N perchloric acid in dioxane VS, determining the endpoint potentiometrically. Perform a blank determination (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 43.24 mg of  $C_{22}H_{26}BrNO_3$ .  
*Acceptance criteria:* 99.0%–100.5% on the dried basis

# IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

# Delete the following:

- **HEAVY METALS** (231)  
*Sample solution:* 1 g in 25 mL of water  
*Acceptance criteria:* NMT 20 ppm (Official 1-Jan-2018)
- **ORGANIC IMPURITIES**  
*Standard solution:* 100 mg/mL of USP Clidinium Bromide RS in 0.1 N methanolic hydrochloric acid  
*Sample solution:* 100 mg/mL of Clidinium Bromide in 0.1 N methanolic hydrochloric acid  
*Reference solution:* Dissolve 100 mg of USP Clidinium Bromide RS in 1.0 mL of 0.1 N methanolic hydrochloric acid, and add 20  $\mu$ L of a solution of 25.0 mg of USP Clidinium Bromide Related Compound A RS in 1.0 mL of 0.1 N methanolic hydrochloric acid.  
**Chromatographic system**  
 (See *Chromatography* (621), *Thin-Layer Chromatography*.)  
*Adsorbent:* 0.25-mm layer of chromatographic silica gel mixture  
*Application volume:* 20  $\mu$ L  
*Developing solvent system:* Acetone, methanol, hydrochloric acid, and water (70:20:5:5)  
*Spray reagent:* Dissolve 850 mg of bismuth subnitrate in a mixture of 10 mL of glacial acetic acid and 40 mL of water. In a separate container, dissolve 20 g of potassium iodide in 50 mL of water. Mix the two solutions, and dilute with dilute sulfuric acid (1 in 10) to 500 mL. Add 7.5 g  $\pm$  2.5 g of iodine, and mix until the solution is complete.  
**Chromatographic plates:** Predevelop suitable thin-layer chromatographic plates by placing in a chromatographic chamber saturated with the *Developing solvent system*, and allow the *Developing solvent system* to move about 15 cm. Remove the plates from the chamber, dry at 105° for 15 min, and cool.  
**Analysis 1 (3-quinuclidinyl benzilate):** Apply the *Standard solution* and the *Sample solution* to a Chromato-

graphic plate. Place the plate in an unsaturated chromatographic chamber containing freshly prepared *Developing solvent system*, and allow the solvent front to move 10 cm. Remove the plate, dry at 105° for 10 min, cool, and spray with potassium iodoplatinate TS.

**Acceptance criteria 1:** The *Sample solution* shows no spot at an  $R_f$  value (about 0.8) corresponding to that of 3-quinuclidinyl benzilate.

**Analysis 2 (limit of clidinium bromide related compound A):** Apply the *Sample solution* and *Reference solution* to a second *Chromatographic plate*. Place the plate in an unsaturated chromatographic chamber containing freshly prepared *Developing solvent system*, and allow the solvent front to move 15 cm. Remove the plate, dry at 105° for 10 min, cool, and spray with the *Spray reagent*.

**Acceptance criteria 2:** Any spot from the *Sample solution* at an  $R_f$  value of about 0.4 is not greater in size or intensity than the minor spot of the *Reference solution*; NMT 0.5% of clidinium bromide related compound A is found.

# SPECIFIC TESTS

- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h; it loses NMT 0.5% of its weight.

# ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
 USP Clidinium Bromide RS  
 USP Clidinium Bromide Related Compound A RS  
 3-Hydroxy-1-methylquinuclidinium bromide.  
 $C_8H_{16}BrNO$  222.13

## Clindamycin Injection

# DEFINITION

Clindamycin Injection contains an amount of Clindamycin Phosphate in Water for Injection equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ). It may be frozen.

# IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

# ASSAY

- **PROCEDURE**  
**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, mix, and filter. Ensure that the concentration of acetonitrile in the *Mobile phase* is NLT 22% and NMT 25% to retain the correct elution order.  
**System suitability stock solution:** 0.1 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*  
**System suitability solution:** 25  $\mu$ g/mL of USP Benzyl Alcohol RS from *System suitability stock solution* and 0.25 mg/mL of USP Clindamycin Phosphate RS, in *Mobile phase*  
**Standard solution:** 0.24 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*  
**Sample stock solution:** Nominally 3 mg/mL of clindamycin from Injection in *Mobile phase*  
**Sample solution:** 0.21 mg/mL of clindamycin from *Sample stock solution* in *Mobile phase*



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 20 µL

**System suitability****Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clindamycin phosphate and benzyl alcohol are 1.0 and 1.2, respectively.]

**Suitability requirements****Resolution:** NLT 2.0 between clindamycin phosphate and benzyl alcohol, *System suitability solution***Relative standard deviation:** NMT 2.5, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) in the portion of the Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

 $r_U$  = peak response of clindamycin phosphate from the *Sample solution* $r_S$  = peak response of clindamycin phosphate from the *Standard solution* $C_S$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL) $P$  = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg) $F$  = conversion factor, 0.001 mg/µg**Acceptance criteria:** 90.0%–120.0%**SPECIFIC TESTS**

- **PH (791):** 5.5–7.0
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.58 USP Endotoxin Unit/mg of clindamycin
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, or in suitable plastic containers.
- **LABELING:** Meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*. Where it is maintained in the frozen state, the label states that it is to be thawed just before use, describes the conditions for proper storage of the resultant solution, and directs that the solution is not to be refrozen.
- **USP REFERENCE STANDARDS (11)**
  - USP Benzyl Alcohol RS
  - USP Clindamycin Phosphate RS
  - USP Endotoxin RS

**Clindamycin for Injection****DEFINITION**Clindamycin for Injection contains an amount of Clindamycin Phosphate equivalent to NLT 758 µg/mg of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ), calculated on the anhydrous basis.**IDENTIFICATION**• **A. INFRARED ABSORPTION (197M)****Sample:** Undried specimen**Acceptance criteria:** Meets the requirements**ASSAY**• **PROCEDURE****Buffer:** Add 14 mL of phosphoric acid to 4000 mL of HPLC grade water. Add 10 mL of ammonium hydroxide, and adjust with ammonium hydroxide to a pH of  $3.90 \pm 0.05$ .**Solution A:** Acetonitrile and methanol (9:1)**Diluent:** *Solution A* and *Buffer* (1:4)**Solution B:** *Solution A* and *Buffer* (2:23)**Solution C:** *Solution A* and *Buffer* (12:13)**Mobile phase:** See *Table 1*.**Table 1**

Time (min)	Solution B (%)	Solution C (%)
0	95	5
40	5	95
41	95	5
46	95	5

**Standard solution:** 2.2 mg/mL of USP Clindamycin Phosphate RS in *Diluent*, prepared as follows. Shake briefly, and sonicate for 5 min to dissolve. Allow to cool to ambient temperature.**Sample solution:** Nominally 2.2 mg/mL of clindamycin phosphate in *Diluent* from Clindamycin for Injection, prepared as follows. Shake briefly, and sonicate for 5 min to dissolve. Allow to cool to ambient temperature.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 25-cm; packing L7

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 25 µL

**System suitability****Sample:** *Standard solution***Suitability requirements****Resolution:** NLT 1.0 between clindamycin phosphate and its nearest related compound. Calculate the resolution using peak widths at half height.**Peak-to-valley ratio:** NLT 2.5

Calculate the peak-to-valley ratio:

$$\text{Result} = H_p/H_v$$

 $H_p$  = height above the baseline of the nearest related compound peak $H_v$  = height above the baseline of the valley between clindamycin phosphate and its nearest related compound**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the quantity of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ), in µg/mg, in the portion of Clindamycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

 $r_U$  = peak area from the *Sample solution* $r_S$  = peak area from the *Standard solution* $C_S$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of clindamycin phosphate in the *Sample solution* (mg/mL) $P$  = potency of clindamycin in the USP Clindamycin Phosphate RS (µg/mg)



Acceptance criteria: NLT 758 µg/mg on the anhydrous basis

### SPECIFIC TESTS

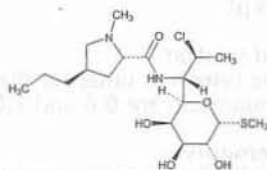
- **BACTERIAL ENDOTOXINS TEST (85):** Contains NMT 0.58 USP Endotoxin Unit/mg of clindamycin
- **STERILITY TESTS (71)**  
 Sample solution: 6 g of Clindamycin for Injection aseptically dissolved in 200 mL of Fluid A  
 Acceptance criteria: Meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*
- **pH (791)**  
 Sample solution: Nominally 10 mg/mL of clindamycin in water from Clindamycin for Injection  
 Acceptance criteria: 3.5–4.5
- **WATER DETERMINATION, Method I (921):** NMT 6.0%
- **CRYSTALLINITY (695):** Meets the requirements
- **OTHER REQUIREMENTS:** It responds to the *Identification test in Clindamycin Phosphate*.

### ADDITIONAL REQUIREMENTS

#### Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements (659), Injection Packaging, Packaging for constitution* (CN 1-May-2017).
- **USP REFERENCE STANDARDS (11)**  
 USP Clindamycin Phosphate RS  
 USP Endotoxin RS

## Clindamycin Hydrochloride



$C_{18}H_{33}ClN_2O_5S \cdot HCl$  461.44  
 $C_{18}H_{33}ClN_2O_5S \cdot HCl \cdot H_2O$  479.47  
*L-threo-α-D-galacto-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidinyl)-carbonyl]amino]-1-thio-, (2S-trans)-, monohydrochloride; Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo-α-D-galacto-octopyranoside monohydrochloride [21462-39-5]. Monohydrate [58207-19-5].*

### DEFINITION

Clindamycin Hydrochloride is the hydrated hydrochloride salt of clindamycin, a substance produced by the chlorination of lincomycin. It has a potency equivalent to NLT 800 µg/mg of  $C_{18}H_{33}ClN_2O_5S$ .

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

Buffer: 6.8 g/L of monobasic potassium phosphate in water. Adjust with 8 N potassium hydroxide to a pH of 7.5.

Mobile phase: Acetonitrile and Buffer (9:11)

Standard solution: 1 mg/mL of USP Clindamycin Hydrochloride RS in *Mobile phase*

Sample solution: 1 mg/mL of Clindamycin Hydrochloride in *Mobile phase*

#### Chromatographic system

(See *Chromatography (621), System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

#### System suitability

Sample: *Standard solution*

#### Suitability requirements

[NOTE—USP Clindamycin Hydrochloride RS contains clindamycin B and 7-epiclindamycin as minor components.]

Resolution: NLT 2.4 between clindamycin B and 7-epiclindamycin and NLT 3.0 between 7-epiclindamycin and clindamycin

Tailing factor: NMT 1.2 for the clindamycin peak

Relative standard deviation: NMT 1.0% for the clindamycin peak

#### Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms for a period of time that is twice the retention time of the clindamycin peak.

Calculate the potency of  $C_{18}H_{33}ClN_2O_5S$ , in µg/mg, in the portion of Clindamycin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak area from the *Sample solution*

$r_S$  = peak area from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clindamycin Hydrochloride in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Hydrochloride RS (µg/mg)

Acceptance criteria: NLT 800 µg/mg

### IMPURITIES

#### Organic Impurities

#### PROCEDURE

Buffer and Mobile phase: Prepare as directed in the *Assay*.

Standard stock solution: 0.5 mg/mL of USP Lincomycin Hydrochloride RS and 1 mg/mL of USP Clindamycin Hydrochloride RS in *Mobile phase*

Standard solution: 50 µg/mL of USP Lincomycin Hydrochloride RS and 100 µg/mL of USP Clindamycin Hydrochloride RS from *Standard stock solution* in *Mobile phase*

Sample solution: 5 mg/mL of Clindamycin Hydrochloride in *Mobile phase*

#### Chromatographic system

(See *Chromatography (621), System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection size: 10 µL

#### Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms for a period of time that is six times the retention time of clindamycin.

Calculate the percentage of lincomycin in the portion of Clindamycin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$



- $r_U$  = peak response of lincomycin from the *Sample solution*  
 $r_S$  = peak response of lincomycin from the *Standard solution*  
 $C_S$  = concentration of USP Lincomycin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Clindamycin Hydrochloride in the *Sample solution* (mg/mL)  
 $P$  = potency of USP Lincomycin Hydrochloride RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
 Calculate the percentage of all other related compounds in the portion of Clindamycin Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- $r_U$  = peak response of each individual related compound, other than lincomycin, from the *Sample solution*  
 $r_S$  = peak response of clindamycin from the *Standard solution*  
 $C_S$  = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of the *Sample solution* (mg/mL)  
 $P$  = potency of USP Clindamycin Hydrochloride RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
 Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Lincomycin <sup>a</sup>	0.4	—
Clindamycin B	0.65	2.0
7-Epiclindamycin	0.8	4.0
Clindamycin	1.0	—
Any other individual related compound	—	1.0
Total related compounds <sup>b</sup>	—	6.0

<sup>a</sup> Lincomycin is controlled in the total of all related compounds. There is no individual acceptance criterion for this compound.

<sup>b</sup> Total of all related compounds including lincomycin.

### SPECIFIC TESTS

- **CRYSTALLINITY (695):** Meets the requirements
- **PH (791):** 3.0–5.5, in a 100-mg/mL solution
- **WATER DETERMINATION, Method 1 (921):** 3.0%–6.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**
  - USP Clindamycin Hydrochloride RS  
*L-threo- $\alpha$ -D-galacto-Octopyranoside*, methyl 7-chloro-6,7,8-trideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidinyl)-carbonyl]amino]-1-thio-, (2*S*-trans)-, monohydrochloride.  
 $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S} \cdot \text{HCl}$  461.45
  - USP Lincomycin Hydrochloride RS  
*D-erythro- $\alpha$ -D-galacto-Octopyranoside*, methyl 6,8-dideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-, monohydrochloride, monohydrate, (2*S*-trans)-.  
 $\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_5\text{S} \cdot \text{HCl} \cdot \text{H}_2\text{O}$  461.02

## Clindamycin Hydrochloride Capsules

### DEFINITION

Clindamycin Hydrochloride Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, relative to the internal standard, as obtained in the Assay.

### ASSAY

#### PROCEDURE

**Mobile phase:** Add 2 g of *dl*-10-camphorsulfonic acid, 1 g of ammonium acetate, and 1 mL of glacial acetic acid to 200 mL of water in a 500-mL volumetric flask, and mix to dissolve. Dilute with methanol to volume, and mix. Adjust, if necessary, with hydrochloric acid or a sodium hydroxide solution (1 in 2) to a pH of  $6.0 \pm 0.1$ .

**Internal standard solution:** Add 0.5 mL of phenylethyl alcohol to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Standard solution:** 18 mg/mL of USP Clindamycin Hydrochloride RS in *Internal standard solution*

**Sample solution:** Equivalent to 15 mg/mL of clindamycin, from the contents of NLT 20 Capsules, in *Internal standard solution*; shake for 30 min; centrifuge or filter, if necessary, to obtain a clear solution.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 4-mm  $\times$  30-cm stainless steel; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 25  $\mu\text{L}$

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for the internal standard and clindamycin are 0.6 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 5.0 between the analyte and internal standard

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ) in the portion of Capsules taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times F \times 100$$

- $R_U$  = peak response ratio of clindamycin to the internal standard from the *Sample solution*  
 $R_S$  = peak response ratio of clindamycin to the internal standard from the *Standard solution*  
 $C_S$  = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)  
 $P$  = potency of clindamycin in USP Clindamycin Hydrochloride RS ( $\mu\text{g}/\text{mg}$ )  
 $F$  = conversion factor, 0.001 mg/ $\mu\text{g}$   
 Acceptance criteria: 90.0%–120.0%

### PERFORMANCE TESTS

#### DISSOLUTION (711)

**Medium:** pH 6.8 phosphate buffer (see *Reagents, Indicators, and Solutions—Buffer Solutions*); 900 mL



**Apparatus 1:** 100 rpm

**Time:** 30 min

**Mobile phase:** Dissolve 16 g of *dl*-10-camphorsulfonic acid, 8 g of ammonium acetate, and 8 mL of glacial acetic acid in 1600 mL of water. Add 2400 mL of methanol, and adjust with hydrochloric acid or 5 N sodium hydroxide to a pH of  $6.0 \pm 0.05$ .

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

**Standard solution:** Prepare a solution of USP Clindamycin Hydrochloride RS in *Medium* having a known concentration similar to that expected in the *Sample solution*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 3- $\mu$ m packing L1

**Flow rate:** 2 mL/min

**Injection size:** 50  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 3.0%

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

Calculate the amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) dissolved.

**Tolerances:** NLT 80% (Q) of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### SPECIFIC TESTS

- **WATER DETERMINATION, Method I (921):** NMT 7.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
USP Clindamycin Hydrochloride RS

## Clindamycin Hydrochloride Oral Solution

#### DEFINITION

Clindamycin Hydrochloride Oral Solution contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ).

#### IDENTIFICATION

- **A.** The retention time of the clindamycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Buffer:** 6.8 g/L of monobasic potassium phosphate in water. Adjust with 8 N potassium hydroxide to a pH of 7.5.

**Mobile phase:** Acetonitrile and *Buffer* (450:550). Increasing the proportion of acetonitrile in the *Mobile phase* decreases the retention time, and decreasing it increases the resolution between 7-epiclindamycin and clindamycin.

**Standard solution:** 1 mg/mL of USP Clindamycin Hydrochloride RS in *Mobile phase*

**Sample solution:** Equivalent to 0.85 mg/mL of clindamycin from Oral Solution in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.4 between the clindamycin B and 7-epiclindamycin peaks, and NLT 3.0 between the 7-epiclindamycin and clindamycin peaks

**Column efficiency:** NLT 4000 theoretical plates from the clindamycin peak

**Tailing factor:** NMT 1.2 for the clindamycin peak

**Relative standard deviation:** NMT 1.0% for the clindamycin peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clindamycin

( $C_{18}H_{33}ClN_2O_5S$ ) in each mL of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- $r_U$  = peak area response from the *Sample solution*  
 $r_S$  = peak area response from the *Standard solution*  
 $C_S$  = concentration of USP Clindamycin Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)  
 $P$  = potency of clindamycin in USP Clindamycin Hydrochloride RS ( $\mu$ g/mg)  
 $F$  = conversion factor, 0.001 mg/ $\mu$ g  
**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for solution packaged in single-unit containers
- **DELIVERABLE VOLUME (698):** Meets the requirements

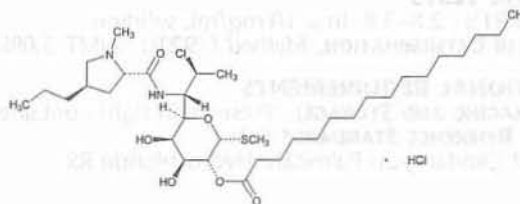
#### SPECIFIC TESTS

- **pH (791):** 2.5–6.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label Oral Solution to indicate that it is intended for veterinary use only.
- **USP REFERENCE STANDARDS (11)**  
USP Clindamycin Hydrochloride RS

## Clindamycin Palmitate Hydrochloride



$C_{34}H_{63}ClN_2O_6S \cdot HCl$  699.85  
*L*-threo- $\alpha$ -D-galacto-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[[(1-methyl-4-propyl-2-pyrrolidine)carbonyl]amino]-1-thio-2-hexadecanoate, monohydrochloride, (2*S*-trans)-;  
 Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-*trans*-4-propyl-2-pyrrolidinecarboxamido)-1-thio-*L*-threo- $\alpha$ -D-galactooctopyranoside 2-palmitate monohydrochloride [25507-04-4].



**DEFINITION**

Clindamycin Palmitate Hydrochloride has a potency equivalent to NLT 540 µg of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ )/mg.

**IDENTIFICATION**• **INFRARED ABSORPTION** (197M)**ASSAY**• **PROCEDURE**

**Mobile phase:** Dissolve 2 g of docusate sodium and 1.54 g of ammonium acetate in a mixture of 2 mL of glacial acetic acid and 75 mL of water. Dilute with methanol to 1 L. Pass through a suitable filter, and degas.

**Standard solution:** 14 mg/mL of USP Clindamycin Palmitate Hydrochloride RS in *Mobile phase*

**Sample solution:** 14 mg/mL of Clindamycin Palmitate Hydrochloride in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Refractive index

**Column:** 3.9-mm × 30-cm; 10-µm packing L1

**Flow rate:** 1.2 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the potency in µg/mg of  $C_{18}H_{33}ClN_2O_5S$  in the portion of Clindamycin Palmitate Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response of clindamycin palmitate from the *Sample solution*

$r_S$  = peak response of clindamycin palmitate from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Palmitate Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of Clindamycin Palmitate Hydrochloride in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Palmitate Hydrochloride RS (µg/mg)

**Acceptance criteria:** NLT 540 µg/mg

**IMPURITIES****Inorganic Impurities**• **RESIDUE ON IGNITION** (281): NMT 0.5%**SPECIFIC TESTS**

• **PH** (791): 2.8–3.8, in a 10 mg/mL solution

• **WATER DETERMINATION, Method I** (921): NMT 3.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers.

• **USP REFERENCE STANDARDS** (11)

USP Clindamycin Palmitate Hydrochloride RS

## Clindamycin Palmitate Hydrochloride for Oral Solution

**DEFINITION**

Clindamycin Palmitate Hydrochloride for Oral Solution is a dry mixture of Clindamycin Palmitate Hydrochloride and one or more suitable buffers, colors, diluents, flavors, and preservatives. It contains the equivalent of NLT 90.0% and

NMT 120.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ), the labeled amount being 15 mg/mL when constituted as directed in the labeling.

**ASSAY**• **PROCEDURE**

**Solution A:** 300 mg/mL of sodium carbonate

**Internal standard solution:** 5 mg/mL of cholesteryl benzoate in chloroform

**Standard solution:** Transfer 150 mg of USP

Clindamycin Palmitate Hydrochloride RS to a glass-stoppered, 15-mL conical centrifuge tube. Add 5 mL of water, 5.0 mL of *Internal standard solution*, and 1 mL of *Solution A*. Insert the stopper, shake vigorously for NLT 10 min, and centrifuge. Remove the upper aqueous layer, and transfer 1.0 mL of the lower chloroform layer to a 15-mL centrifuge tube. Add 1.0 mL of pyridine and 1.0 mL of acetic anhydride. Agitate the tube to ensure complete mixing, cover the top of the centrifuge tube with a plastic cap through which a small hole has been punched, heat at 100° for 2.5 h, and allow to cool. Mix, and centrifuge if necessary. Use the clear solution.

**Sample solution:** Constitute the Clindamycin Palmitate Hydrochloride for Oral Solution as directed in the labeling, and transfer 5.0 mL of the constituted solution to a glass-stoppered, 15-mL conical centrifuge tube. Add 5.0 mL of *Internal standard solution* and 1 mL of *Solution A*. Insert the stopper, shake vigorously for NLT 10 min, and centrifuge. Remove the upper aqueous layer, and transfer 1.0 mL of the lower chloroform layer to a 15-mL centrifuge tube. Add 1.0 mL of pyridine and 1.0 mL of acetic anhydride. Agitate the tube to ensure complete mixing, cover the top of the centrifuge tube with a plastic cap through which a small hole has been punched, heat at 100° for 2.5 h, and allow to cool. Mix, and centrifuge if necessary. Use the clear solution.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.6-m × 3-mm glass; packing 1% phase G36 on support S1AB

**Temperature**

**Column:** 290°

**Detector:** 320°

**Carrier gas:** Dry helium

**Flow rate:** 60 mL/min

**Injection size:** 1.0 µL

**System suitability**

**Sample:** *Standard solution*

The elution order is: cholesteryl benzoate, clindamycin palmitate.

**Suitability requirements:** In a suitable chromatogram, the peaks are completely resolved.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) in each mL of the solution constituted from Clindamycin Palmitate Hydrochloride for Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times P \times 100$$

$R_U$  = internal standard ratio (peak response of clindamycin palmitate/peak response of cholesteryl benzoate) from the *Sample solution*

$R_S$  = internal standard ratio (peak response of clindamycin palmitate/peak response of cholesteryl benzoate) from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Palmitate Hydrochloride RS in the *Standard solution* (mg/mL)



- $C_u$  = nominal concentration of clindamycin palmitate hydrochloride in the *Sample solution* (mg/mL)  
 $P$  = potency of clindamycin in USP Clindamycin Palmitate Hydrochloride RS ( $\mu\text{g}/\text{mg}$ )  
 Acceptance criteria: 90.0%–120.0%

**PERFORMANCE TESTS**

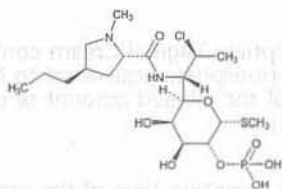
- UNIFORMITY OF DOSAGE UNITS** (905)  
For solids packaged in single-unit containers:  
Meets the requirements
- DELIVERABLE VOLUME** (698): Meets the requirements

**SPECIFIC TESTS**

- PH** (791): 2.5–5.0, in the solution constituted as directed in the labeling
- WATER DETERMINATION, Method I** (921): NMT 3.0%

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers.
- USP REFERENCE STANDARDS** (11)  
USP Clindamycin Palmitate Hydrochloride RS

**Clindamycin Phosphate**

$\text{C}_{18}\text{H}_{34}\text{ClN}_2\text{O}_8\text{P}$  504.96  
 L-threo- $\alpha$ -D-galacto-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[[1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-, 2-(dihydrogen phosphate), (2S-trans)-;

Methyl 7-chloro-6,7,8-trideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-L-threo- $\alpha$ -D-galacto-octopyranoside 2-(dihydrogen phosphate) [24729-96-2].

**DEFINITION**

Clindamycin Phosphate has a potency equivalent to NLT 758  $\mu\text{g}/\text{mg}$  of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ), calculated on the anhydrous basis.

**IDENTIFICATION**

- A. INFRARED ABSORPTION** (197K)  
Standard: Add 0.2 mL of water to 50 mg of USP Clindamycin Phosphate RS, and heat to dissolve. Evaporate to dryness under vacuum, and dry the residue at 100°–105° for 2 h.  
Sample: Add 0.2 mL of water to 50 mg of Clindamycin Phosphate, and heat to dissolve. Evaporate to dryness under vacuum, and dry the residue at 100°–105° for 2 h.  
Acceptance criteria: Meets the requirements
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

- PROCEDURE**  
Solution A: Add 14 mL of phosphoric acid to 4000 mL of water. Add 10 mL of ammonium hydroxide, and adjust with ammonium hydroxide to a pH of  $5.6 \pm 0.1$ .

Solution B: Acetonitrile and methanol (900:100)  
 Solution C: *Solution B* and *Solution A* (80:920)  
 Solution D: *Solution B* and *Solution A* (480:520)  
 Diluent: *Solution B* and *Solution A* (20:80)  
 Mobile phase: See Table 1.

Table 1

Time (min)	Solution C (%)	Solution D (%)
0	95	5
40	5	95
41	95	5
46	95	5

Standard solution: 2.2 mg/mL of USP Clindamycin Phosphate RS in *Diluent*. Shake, and sonicate to dissolve.

Sample solution: 2.2 mg/mL of Clindamycin Phosphate in *Diluent*. Shake, and sonicate to dissolve.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm  $\times$  25-cm; 5- $\mu\text{m}$  packing L7

Column temperature: 40°

Flow rate: 1.2 mL/min

Injection volume: 20  $\mu\text{L}$

**System suitability**

Sample: *Standard solution*

[NOTE—USP Clindamycin Phosphate RS contains 7-epiclindamycin phosphate. See Table 2 for the relative retention times.]

**Suitability requirements**

Resolution: NLT 3.0 between clindamycin phosphate and 7-epiclindamycin phosphate

Tailing factor: NMT 2.0 for clindamycin phosphate

Relative standard deviation: NMT 0.73%

**Analysis**

Samples: *Standard solution* and *Sample solution*

Calculate the quantity of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ), in  $\mu\text{g}/\text{mg}$ , in the portion of Clindamycin Phosphate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Clindamycin Phosphate in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Phosphate RS ( $\mu\text{g}/\text{mg}$ )

Acceptance criteria: NLT 758  $\mu\text{g}/\text{mg}$  on the anhydrous basis

**IMPURITIES****ORGANIC IMPURITIES**

Solution A, Solution B, Solution C, Solution D, Diluent, Mobile phase, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

System suitability solution: 2.2 mg/mL of USP Clindamycin Phosphate RS in *Diluent*. Shake, and sonicate to dissolve.

Standard solution: 14  $\mu\text{g}/\text{mL}$  of Clindamycin Phosphate from *System suitability solution* in *Diluent*

**System suitability**

Samples: *System suitability solution* and *Standard solution*

[NOTE—USP Clindamycin Phosphate RS contains 7-epiclindamycin phosphate. See Table 2 for the relative retention times.]



**Suitability requirements**

**Resolution:** NLT 3.0 between 7-epiclindamycin phosphate and clindamycin phosphate, *System suitability solution*

**Tailing factor:** NMT 2.0 for clindamycin phosphate, *Standard solution*

**Relative standard deviation:** NMT 5.0% for clindamycin phosphate, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of each impurity in the portion of Clindamycin Phosphate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of clindamycin phosphate from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clindamycin Phosphate, corrected for water content, in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Phosphate RS ( $\mu\text{g}/\text{mg}$ )

$F_1$  = conversion factor, 0.001 mg/ $\mu\text{g}$

$F_2$  = relative response factor (see Table 2)

**Acceptance criteria:** See Table 2. The reporting level is 0.05%.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Lincomycin phosphate <sup>a</sup>	0.36	1.0	1.0
Lincomycin <sup>b</sup>	0.50	2.0	0.5
Clindamycin B phosphate <sup>c</sup>	0.77	1.0	1.5
7-Epiclindamycin phosphate <sup>d</sup>	0.89	1.0	0.8
Clindamycin 3-phosphate <sup>e</sup>	0.93	1.0	0.3
Clindamycin phosphate	1.0	—	—
Clindamycin <sup>f</sup>	1.4	1.0	0.5
Any individual, unspecified impurity	—	1.0	1.0
Total impurities	—	—	4.0

<sup>a</sup> Methyl 6,8-dideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-erythro- $\alpha$ -D-galacto-octopyranoside 2-phosphate.

<sup>b</sup> Methyl 6,8-dideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-erythro- $\alpha$ -D-galacto-octopyranoside.

<sup>c</sup> Methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-1-methyl-4-ethylpyrrolidine-2-carboxamido]-1-thio-L-threo- $\alpha$ -D-galacto-octopyranoside 2-phosphate.

<sup>d</sup> Methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-erythro- $\alpha$ -D-galacto-octopyranoside 2-phosphate.

<sup>e</sup> Methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-1-methyl-4-ethylpyrrolidine-2-carboxamido]-1-thio-L-threo- $\alpha$ -D-galacto-octopyranoside 3-phosphate.

<sup>f</sup> Methyl 7-chloro-6,7,8-trideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-threo- $\alpha$ -D-galacto-octopyranoside.

**SPECIFIC TESTS**

• **CRYSTALLINITY** (695): Meets the requirements

• **pH** (791)

*Sample solution:* 10 mg/mL

*Acceptance criteria:* 3.5–4.5

• **WATER DETERMINATION, Method I** (921): NMT 6.0%

• **STERILITY TESTS** (71)

*Sample solution:* 6 g of specimen aseptically dissolved in 200 mL of Fluid A

**Analysis:** Test as directed in the *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

**Acceptance criteria:** It meets the requirements where the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.58 USP Endotoxin Unit/mg of clindamycin, where the label states that Clindamycin Phosphate is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store below 30°.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.
- **USP REFERENCE STANDARDS** (11)  
USP Clindamycin Phosphate RS  
USP Endotoxin RS

**Clindamycin Phosphate Vaginal Cream****DEFINITION**

Clindamycin Phosphate Vaginal Cream contains an amount of clindamycin phosphate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ( $\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$ ).

**IDENTIFICATION**

- **A.** The relative retention time of the major peak for clindamycin phosphate of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, and mix.

**System suitability solution:** 0.6 mg/mL each of USP Clindamycin Phosphate RS and USP Clindamycin Hydrochloride RS in *Mobile phase*

**Standard solution:** 0.25 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*

**Sample solution:** Nominally 0.2 mg/mL of clindamycin in *Mobile phase* from Cream, prepared as follows. Transfer a suitable portion of Cream to a stoppered conical flask, and add *Mobile phase*. Add about 10 glass beads (about 10 mm in diameter). Insert the stopper securely in the flask, and shake by mechanical means at 50° for 1 h. Cool in an ice bath for 20 min, and centrifuge. Pass a portion of the cloudy lower layer through a filter of 2- $\mu\text{m}$  or finer pore size, and use the filtrate.

**Chromatographic system**

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu\text{L}$

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clindamycin phosphate and clindamycin are about 1.0 and 1.5, respectively.]



**Suitability requirements**

**Resolution:** NLT 6.0 between clindamycin phosphate and clindamycin, *System suitability solution*

**Column efficiency:** NLT 1700 theoretical plates, *System suitability solution*

**Tailing factor:** NMT 1.3, *System suitability solution*

**Relative standard deviation:** NMT 2.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Phosphate RS ( $\mu\text{g}/\text{mg}$ )

$F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- PH (791):** 3.0–6.0, determined on the undiluted Cream

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in well-closed containers.
- USP REFERENCE STANDARDS (11)**  
USP Clindamycin Hydrochloride RS  
USP Clindamycin Phosphate RS

**Clindamycin Phosphate Gel****DEFINITION**

Clindamycin Phosphate Gel contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ).

**IDENTIFICATION**

- A.** The retention time of the clindamycin phosphate peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE**

**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, and mix.

**System suitability solution:** 0.6 mg/mL each of USP Clindamycin Phosphate RS and USP Clindamycin Hydrochloride RS in *Mobile phase*

**Standard solution:** 0.25 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*

**Sample solution:** Nominally 0.2 mg/mL of clindamycin in *Mobile phase* from Gel. Shake by mechanical means for 30 min. Centrifuge a portion of the solution, and if necessary, filter a portion of the supernatant. Use the clear filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu\text{L}$

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clindamycin phosphate and clindamycin are 1.0 and 1.5, respectively.]

**Suitability requirements**

**Resolution:** NLT 6.0 between the clindamycin phosphate and clindamycin peaks, *System suitability solution*

**Column efficiency:** NLT 1700 theoretical plates, *System suitability solution*

Calculate as follows:

$$\text{Result} = (t_r/W_{h/2})^2 \times 5.545$$

$t_r$  = retention time

$W_{h/2}$  = peak width at half height

**Tailing factor:** NMT 1.3, *System suitability solution*

**Relative standard deviation:** NMT 2.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) in the portion of Gel taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Phosphate RS ( $\mu\text{g}/\text{mg}$ )

$F$  = conversion factor, 0.001 mg/ $\mu\text{g}$

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- MINIMUM FILL (755):** Meets the requirements

**SPECIFIC TESTS**

- PH (791):** 4.5–6.5

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers.
- USP REFERENCE STANDARDS (11)**  
USP Clindamycin Hydrochloride RS  
USP Clindamycin Phosphate RS

**Clindamycin Phosphate Topical Solution****DEFINITION**

Clindamycin Phosphate Topical Solution contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.



**ASSAY****• PROCEDURE**

**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, mix, and filter. Ensure that the concentration of acetonitrile in the *Mobile phase* is NLT 22% and NMT 25% to retain the correct elution order.

**System suitability stock solution 1:** 4 mg/mL of 4'-hydroxyacetophenone in acetonitrile

**System suitability stock solution 2:** 0.04 mg/mL of 4'-hydroxyacetophenone from *System suitability stock solution 1* in *Mobile phase*

**Standard solution:** 0.24 mg/mL of USP Clindamycin Phosphate RS in *Mobile phase*

**System suitability solution:** Mix 1 part of *System suitability stock solution 2* with 3 parts of *Standard solution*.

**Sample solution:** Equivalent to 0.2 mg/mL of clindamycin from Topical Solution in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clindamycin phosphate and 4'-hydroxyacetophenone are about 1.0 and 1.2, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between clindamycin phosphate and 4'-hydroxyacetophenone, *System suitability solution*

**Relative standard deviation:** NMT 2.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) in the portion of the Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- pH (791):** 4.0–7.0

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers.
- USP REFERENCE STANDARDS (11)**  
USP Clindamycin Phosphate RS

## Clindamycin Phosphate Topical Suspension

**DEFINITION**

Clindamycin Phosphate Topical Suspension contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ).

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY****• PROCEDURE**

**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, mix, and filter.

**System suitability solution:** 0.6 mg/mL each of USP Clindamycin Phosphate RS and USP Clindamycin Hydrochloride RS, in the *Mobile phase*

**Standard solution:** 0.25 mg/mL of USP Clindamycin Phosphate RS in the *Mobile phase*

**Sample solution:** Equivalent to 0.2 mg/mL of clindamycin from Topical Suspension in *Mobile phase*. Prepare as follows. Using a suitable hypodermic needle and syringe, transfer a suitable aliquot of Topical Suspension to a suitable volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clindamycin phosphate and clindamycin are about 1.0 and 1.5, respectively.]

**Suitability requirements**

**Resolution:** NLT 6.0 between the clindamycin phosphate and clindamycin peaks, *System suitability solution*

**Column efficiency:** NLT 1700 theoretical plates, *System suitability solution*, calculated from the peak width at half height

**Tailing factor:** NMT 1.3, *System suitability solution*

**Relative standard deviation:** NMT 2.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) in the portion of the Topical Suspension taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in USP Clindamycin Phosphate RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg



Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

#### SPECIFIC TESTS

- **PH (791):** 4.5–6.5

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS (11)**  
USP Clindamycin Hydrochloride RS  
USP Clindamycin Phosphate RS

### Clindamycin Phosphate Vaginal Inserts

#### DEFINITION

Clindamycin Phosphate Vaginal Inserts contain the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ).

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**

**Sample:** Transfer a Vaginal Insert into a suitable container, add 120 mL of methylene chloride, insert a stopper, and shake until the Vaginal Insert is completely dissolved. Using a vacuum, pass through a methylene chloride-compatible filter having a 0.45- $\mu$ m pore size. Rinse the filter with several portions of methylene chloride, and allow the filter to air-dry. Use the white residue to prepare the mineral oil dispersion for the test.

**Acceptance criteria:** The IR absorption of the *Sample* exhibits maxima at the same wavelengths as that of a similar preparation of USP Clindamycin Phosphate RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

- **PROCEDURE**

**Buffer:** 10.54 g/L of monobasic potassium phosphate in water. Adjust with phosphoric acid to a pH of 2.5.

**Mobile phase:** Dissolve 10.54 g of monobasic potassium phosphate in 775 mL of water, and adjust with phosphoric acid to a pH of 2.5. Add 225 mL of acetonitrile, and mix.

**System suitability solution:** 0.24 mg/mL of USP Clindamycin Phosphate RS and 6  $\mu$ g/mL of USP Clindamycin Hydrochloride RS, in *Buffer*

**Standard solution:** 0.24 mg/mL of USP Clindamycin Phosphate RS in *Buffer*

**Sample solution:** Transfer 1 Vaginal Insert to a suitable 100-mL container. Add 40 mL of isooctane, and seal the container tightly with a teflon-lined septum and crimp cap. Shake vigorously for about 15 min until all of the Vaginal Insert is dissolved. Add 40.0 mL of *Buffer*. Recap the container tightly, and shake vigorously for NLT 30 min, taking care to avoid leakage. Allow the layers to separate, and remove a volume of the lower aqueous layer sufficient to perform the following steps. Pass the aqueous solution through a filter having a 5- $\mu$ m or finer pore size, discarding the first 2 mL of the filtrate. Collect the remaining filtrate, and prepare a solution equivalent to 0.2 mg/mL of clindamycin with *Buffer*.

#### Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm  $\times$  25-cm; packing L7

Flow rate: 1 mL/min

Injection volume: 35  $\mu$ L

System suitability

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between clindamycin phosphate and clindamycin hydrochloride, *System suitability solution*

Calculate as follows.

$$\text{Result} = [(t_2 - t_1)/(w_{h1} + w_{h2})] \times 1.177$$

$t_2$  = retention time of the second peak

$t_1$  = retention time of the first peak

$w_{h1}$  = height at half width of the first peak

$w_{h2}$  = height at half width of the second peak

**Relative standard deviation:** NMT 2.5%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clindamycin ( $C_{18}H_{33}ClN_2O_5S$ ) equivalent in the Vaginal Insert taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Clindamycin Phosphate RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of clindamycin in the *Sample solution* (mg/mL)

$P$  = potency of clindamycin in the USP Clindamycin Phosphate RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

**Acceptance criteria:** 90.0%–110.0%. Use as the Assay value the average of the determinations obtained in the test for *Uniformity of Dosage Units* (905), *Content Uniformity*.

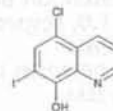
#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers, at controlled room temperature, or in a cool place.
- **USP REFERENCE STANDARDS (11)**  
USP Clindamycin Hydrochloride RS  
USP Clindamycin Phosphate RS

### Clioquinol



$C_9H_5ClINO$

305.50

8-Quinololinol, 5-chloro-7-iodo-;  
5-Chloro-7-iodo-8-quinolinol [130-26-7].

#### DEFINITION

Clioquinol, dried over phosphorus pentoxide for 5 h, contains NLT 93.0% and NMT 100.5% of clioquinol ( $C_9H_5ClINO$ ).



**IDENTIFICATION**

- **A.**  
**Standard solution:** Prepare as directed for the *Standard solution* in the *Assay*, except use 1.0 mL of pyridine instead of the *Internal standard solution*.  
**Acceptance criteria:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. ULTRAVIOLET ABSORPTION (197U)**  
**Analytical wavelength:** 267 nm  
**Medium:** 3 N hydrochloric acid  
**Sample solution:** 5 µg/mL  
**Acceptance criteria:** Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.
- **C.**  
**Sample:** 100 mg  
**Analysis:** Heat the *Sample* with 5 mL of sulfuric acid.  
**Acceptance criteria:** Copious violet vapors of iodine are evolved.

**ASSAY**• **PROCEDURE**

**Internal standard solution:** 2 mg/mL of pyrene in pyridine  
**Standard stock solution:** 3 mg/mL of USP Clioquinol RS in a mixture of pyridine and *n*-hexane (4:1)  
**Standard solution:** Transfer 1.0 mL of the *Standard stock solution* to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, attach the cap, and mix. Heat in a water bath at 50° for 15 min, and then cool to ambient temperature.

**Sample stock solution:** 3 mg/mL of Clioquinol, previously dried, in a mixture of pyridine and *n*-hexane (4:1)

**Sample solution:** Transfer 1.0 mL of the *Sample stock solution* to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, and attach the cap. Heat in a water bath at 50° for 15 min, and then cool to ambient temperature.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 1.83-m × 2-mm glass; packed with 3% liquid phase G3 on 80- to 100-mesh support S1AB

**Temperatures**

**Column:** The initial temperature is 200° for a conditioning period of NLT 16 h (not connected to the detector) and is then reduced to 165°.

**Injection port:** 170°

**Detector:** 250°

**Carrier gas:** Helium

**Flow rate:** 30 mL/min for helium. Hydrogen and air are introduced into the detector at rates of 25 and 500 mL/min, respectively.

**Injection volume:** 1 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for clioquinol and pyrene are 0.6 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between the clioquinol and the internal standard peaks

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clioquinol (C<sub>9</sub>H<sub>5</sub>ClINO) in the portion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of clioquinol to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of clioquinol to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Clioquinol RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clioquinol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–100.5%

**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 0.5%

**SPECIFIC TESTS**

- **LOSS ON DRYING (731)**

**Analysis:** Dry a sample over phosphorus pentoxide for 5 h.

**Acceptance criteria:** NMT 0.5%

- **FREE IODINE AND IODIDE**

**Control solution:** Dilute 2.0 mL of potassium iodide solution (1 in 6000) with water to 10 mL, add 6 mL of 2 N sulfuric acid, 1 mL of potassium dichromate TS, and 2 mL of chloroform, and shake for 15 s (0.05% of iodide).

**Sample:** 1.0 g

**Analysis:** Shake the *Sample* with 20 mL of water for 30 s, allow to stand for 5 min, and filter. To 10 mL of the filtrate add 1 mL of 2 N sulfuric acid, then add 2 mL of chloroform, and shake; no violet color appears in the chloroform (free iodine). To the mixture add 5 mL of 2 N sulfuric acid and 1 mL of potassium dichromate TS, and shake for 15 s.

**Acceptance criteria:** The color of the chloroform layer is no deeper than that produced in the *Control solution*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
 USP Clioquinol RS

**Clioquinol Cream****DEFINITION**

Clioquinol Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of clioquinol (C<sub>9</sub>H<sub>5</sub>ClINO) in a suitable cream base.

**IDENTIFICATION**

- **A.**  
**Standard solution:** Prepare as directed for the *Standard solution* in the *Assay*, except use 1.0 mL of pyridine instead of the *Internal standard solution*.  
**Acceptance criteria:** The retention time of the major peak of the *Sample solution*, as obtained in the *Assay*, corresponds to that of the *Standard solution*.
- **B.**  
**Sample solution:** Place nominally 25 mg of clioquinol in a 100-mL volumetric flask, add 75 mL of dilute hydrochloric acid (1 in 4), and heat on a steam bath to melt the Cream, shaking vigorously to extract the clioquinol. Cool under running water, and add dilute hydrochloric acid (1 in 4) to volume. Filter through paper, and dilute 3 mL of the filtrate with dilute hydrochloric acid (1 in 4) to 100 mL.  
**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Clioquinol RS, concomitantly measured.

**ASSAY**• **PROCEDURE**

**Internal standard solution:** 2 mg/mL of pyrene in pyridine

**Standard stock solution:** 3 mg/mL of USP Clioquinol RS in a mixture of pyridine and *n*-hexane (4:1)



**Standard solution:** Transfer 1.0 mL of the *Standard stock solution* to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, attach the cap, and mix. Heat in a water bath at 50° for 15 min, and then cool to ambient temperature.

**Sample stock solution:** Transfer nominally 150 mg of clioquinol from Cream to a 60-mL separator. Place the separator on its side in a vacuum oven at a pressure of 10 mm of mercury at 45° for 4 h. Remove the separator from the oven, allow to cool, add 15 mL of a mixture of pyridine and *n*-hexane (4:1), and insert a polytetrafluoroethylene stopper. Transfer the mixture to a 50-mL volumetric flask, and rinse the separator with two 15-mL portions of the same solvent, shaking each time for 30 s. Transfer both rinsings to the volumetric flask, and dilute with the same solvent to volume.

**Sample solution:** Transfer 1.0 mL of the *Sample stock solution* to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, and attach the cap. Heat in a water bath at 50° for 15 min, and then cool to ambient temperature.

**Chromatographic system**  
(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 1.83-m × 2-mm glass; packed with 3% liquid phase G3 on 80- to 100-mesh support S1AB

**Temperatures**

**Column:** The initial temperature is 200° for a conditioning period of NLT 16 h (not connected to the detector) and is then reduced to 165°.

**Injection port:** 170°

**Detector:** 250°

**Carrier gas:** Helium

**Flow rate:** 30 mL/min for helium. Hydrogen and air are introduced into the detector at rates of 25 and 500 mL/min, respectively.

**Injection volume:** 1 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for clioquinol and pyrene are 0.6 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 3 between the clioquinol and the internal standard peaks

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clioquinol ( $C_9H_5ClINO$ ) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of clioquinol to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of clioquinol to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Clioquinol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clioquinol in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight, light-resistant containers.

- **USP REFERENCE STANDARDS (11)**  
USP Clioquinol RS

## Clioquinol Ointment

### DEFINITION

Clioquinol Ointment contains NLT 90.0% and NMT 110.0% of the labeled amount of clioquinol ( $C_9H_5ClINO$ ) in a suitable ointment base.

### IDENTIFICATION

- **A.**  
**Standard solution:** Prepare as directed for the *Standard solution* in the *Assay*, except use 1.0 mL of pyridine instead of the *Internal standard solution*.  
**Acceptance criteria:** The retention time of the major peak of the *Sample solution*, as obtained in the *Assay*, corresponds to that of the *Standard solution*.

- **B.**  
**Sample solution:** Place nominally 25 mg of clioquinol from Ointment in a 100-mL volumetric flask, add 75 mL of dilute hydrochloric acid (1 in 4), and heat on a steam bath to melt the Ointment, shaking vigorously to extract the clioquinol. Cool under running water, and add dilute hydrochloric acid (1 in 4) to volume. Filter through paper, and dilute 3 mL of the filtrate with dilute hydrochloric acid (1 in 4) to 100 mL.  
**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Clioquinol RS, concomitantly measured.

### ASSAY

- **PROCEDURE**  
**Internal standard solution:** 2 mg/mL of pyrene in pyridine  
**Standard stock solution:** 3 mg/mL of USP Clioquinol RS in a mixture of pyridine and *n*-hexane (4:1)  
**Standard solution:** Transfer 1.0 mL of the *Standard stock solution* to a screw-capped glass vial fitted with a septum, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, and attach the cap. Heat in a water bath at 50° for 15 min, and then cool to ambient temperature.  
**Sample solution:** Transfer nominally 150 mg of clioquinol from Ointment to a 125-mL separator. Add 75 mL of *n*-hexane, then add 15 mL of dimethylformamide, and mix for 1 min. Allow the layers to separate, and transfer the lower layer to a 50-mL volumetric flask. Repeat the extraction with separate 15- and 10-mL portions of dimethylformamide, and transfer the lower layers to the 50-mL volumetric flask. Dilute with dimethylformamide to volume. Transfer 1.0 mL of this solution to a screw-capped glass vial fitted with a septum, and evaporate at 60° under a stream of nitrogen to dryness. Add 1.0 mL of a mixture of pyridine and *n*-hexane (4:1) to the residue, add 1.0 mL of bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution*, and attach the cap. Heat in a water bath at 50° for 15 min, then cool to ambient temperature.

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 1.83-m × 2-mm glass; packed with 3% liquid phase G3 on 80- to 100-mesh support S1AB

**Temperatures**

**Column:** The initial temperature is 200° for a conditioning period of NLT 16 h (not connected to the detector) and is then reduced to 165°.



Injection port: 170°  
 Detector: 250°  
 Carrier gas: Helium  
 Flow rate: 30 mL/min for helium. Hydrogen and air are introduced into the detector at rates of 25 and 500 mL/min, respectively.

Injection volume: 1 µL

#### System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for clioquinol and pyrene are 0.6 and 1.0, respectively.]

#### Suitability requirements

Resolution: NLT 3 between the clioquinol and the internal standard peaks

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clioquinol ( $C_9H_5ClINO$ ) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of clioquinol to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of clioquinol to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Clioquinol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clioquinol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Clioquinol RS

### Compound Clioquinol Topical Powder

#### DEFINITION

Compound Clioquinol Topical Powder contains NLT 22.5% and NMT 27.5% of clioquinol ( $C_9H_5ClINO$ ).

Clioquinol	250 g
Lactic Acid	25 g
Zinc Stearate	200 g
Lactose	525 g
To make	1000 g

Mix the *Lactic Acid* with *Lactose*. Then add *Clioquinol* and *Zinc Stearate* to the mixture, and mix.

#### IDENTIFICATION

- **A.** Sample: A quantity of Topical Powder equivalent to 30 mg of clioquinol  
 Analysis: Place the *Sample* in a glass-stoppered, 50-mL conical flask, add 20 mL of 1 N sulfuric acid, and shake for 5 min. Filter, and transfer 5 mL of the filtrate to a glass-stoppered test tube. Add 5 drops of potassium dichromate TS and 2 mL of chloroform, and shake well.  
 Acceptance criteria: A red-violet color develops in the chloroform layer.

#### ASSAY

##### PROCEDURE

**Standard solution:** 5 µg/mL of USP Clioquinol RS in 3 N hydrochloric acid

**Sample solution:** Transfer a quantity of Topical Powder equivalent to 50 mg of clioquinol to a 200-mL volumetric flask. Add 100 mL of 3 N hydrochloric acid, and

shake by mechanical means for 15 min. Dilute with 3 N hydrochloric acid to volume. Filter a portion of the solution. Dilute 4.0 mL of the filtrate with 3 N hydrochloric acid to 200.0 mL, and mix.

**Blank:** 3 N hydrochloric acid

#### Instrumental conditions

Mode: UV

Analytical wavelength: 267 nm

Cell: 1 cm

#### Analysis

Samples: *Standard solution* and *Sample solution*

Determine the absorbances of the *Standard solution* and the *Sample solution* at the wavelength of maximum absorbance.

Calculate the percentage of clioquinol ( $C_9H_5ClINO$ ) in the portion of Topical Powder taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of clioquinol in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of clioquinol in the *Sample solution* (µg/mL)

Acceptance criteria: 22.5%–27.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Clioquinol RS

### Clioquinol and Hydrocortisone Cream

#### DEFINITION

Clioquinol and Hydrocortisone Cream contains NLT 90.0% and NMT 110.0% of the labeled amounts of clioquinol ( $C_9H_5ClINO$ ) and hydrocortisone ( $C_{21}H_{30}O_5$ ) in a suitable cream base.

#### IDENTIFICATION

- **A.** The retention time of the clioquinol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Clioquinol*.
- **B.** The retention time of the hydrocortisone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay for Hydrocortisone*.

#### ASSAY

##### CLIOQUINOL

**Internal standard solution:** 2 mg/mL of pyrene in pyridine

**Standard stock solution:** 3 mg/mL of USP Clioquinol RS in a mixture of pyridine and *n*-hexane (4:1)

**Standard solution:** Transfer 1.0 mL of the *Standard stock solution*, 1.0 mL of *N,O*-bis(trimethylsilyl)acetamide, and 1.0 mL of *Internal standard solution* to a suitable screw-capped glass vial fitted with a polytetrafluoroethylene septum, and mix. Heat on a water bath at 50° for 15 min, and cool to room temperature.

**Sample solution:** Transfer nominally 150 mg of clioquinol from Cream to a 60-mL separator. Place the separator on its side in a vacuum oven at 45° for 4 h. Remove the separator, cool to room temperature, and add 15.0 mL of a mixture of pyridine and hexane (4:1). Insert the stopper in the separator, and mix until the specimen is completely dispersed. Transfer the contents of the separator to a 50-mL volumetric flask, rinse the separator with two 15-mL portions of a mixture of pyridine and hexane (4:1), collecting the rinsings in the volumetric flask, and dilute with the same solvent mix-



ture to volume. Immediately transfer 1 mL of this solution to a dry, screw-capped glass vial, and evaporate with the aid of gentle heat and a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of a mixture of pyridine and hexane (4:1), add 1 mL each of *N,O*-bis(trimethylsilyl)acetamide and *Internal standard solution* to the screw-capped glass vial fitted with a polytetrafluoroethylene septum, and mix. Heat on a water bath at 50° for 15 min, and cool to room temperature.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m; packed with 3% liquid phase G3 on 80- to 100-mesh support SIAB

Temperatures

Column: 165°

Injection port: 170°

Detector: 250°

Carrier gas: Dry helium

Flow rate: 30 mL/min

Injection volume: 1 µL

#### System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for clioquinol and pyrene are 0.6 and 1.0, respectively.]

#### Suitability requirements

Resolution: NLT 3.0 between the analyte and internal standard peaks

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms to obtain NLT 40% of maximum recorder response, and measure the peak response of each component.

Calculate the percentage of the labeled amount of clioquinol ( $C_9H_5ClINO$ ) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of clioquinol to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of clioquinol to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Clioquinol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clioquinol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### • HYDROCORTISONE

Mobile phase: Acetonitrile, methanol, and water (1:1:2.75)

Standard stock solution: 1 mg/mL of USP Hydrocortisone RS in alcohol

Standard solution: 100 µg/mL of USP Hydrocortisone RS in alcohol from the *Standard stock solution*

Sample solution: Transfer nominally 10 mg of hydrocortisone from Cream to a 50-mL centrifuge tube. Add 30 mL of alcohol, and heat on a steam bath just to boiling. Shake for 15 min, and centrifuge. Transfer the supernatant extract to a 100-mL volumetric flask. Repeat the extraction with two 20-mL portions of alcohol, combining the extracts in the 100-mL volumetric flask. Add alcohol to volume, mix, and filter.

System suitability stock solution: 0.5 mg/mL of methylparaben in alcohol

System suitability solution: Transfer 2 mL of *System suitability stock solution* and 20 mL of *Standard stock solution* into a 200-mL volumetric flask, and dilute with alcohol to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Columns

Guard: Packing L2

Analytical: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

#### System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for methylparaben and hydrocortisone are 0.6 and 1.0, respectively.]

#### Suitability requirements

Resolution: NLT 2.0 between the hydrocortisone and methylparaben peaks

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone ( $C_{21}H_{30}O_5$ ) in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL** (755): Meets the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
  - USP Clioquinol RS
  - USP Hydrocortisone RS

## Clioquinol and Hydrocortisone Ointment

#### DEFINITION

Clioquinol and Hydrocortisone Ointment contains NLT 90.0% and NMT 110.0% of the labeled amounts of clioquinol ( $C_9H_5ClINO$ ) and hydrocortisone ( $C_{21}H_{30}O_5$ ) in a suitable ointment base.

#### IDENTIFICATION

- **A.** The retention time of the clioquinol peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for Clioquinol.
- **B.** The retention time of the hydrocortisone peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for Hydrocortisone.

#### ASSAY

##### • CLIOQUINOL

Internal standard solution: 2 mg/mL of pyrene in pyridine

Standard stock solution: 3 mg/mL of USP Clioquinol RS in a mixture of pyridine and *n*-hexane (4:1)

Standard solution: Transfer 1.0 mL of the *Standard stock solution*, 1.0 mL of *N,O*-bis(trimethylsilyl)acetamide, and 1.0 mL of *Internal standard solution* to a suitable screw-capped glass vial fitted with a polytetrafluoroethylene septum, and mix. Heat on a water bath at 50° for 15 min, and cool to room temperature.

Sample solution: Transfer nominally 150 mg of clioquinol from Ointment to a 125-mL separator. Add 75 mL of *n*-hexane, insert the stopper in the separator, and



mix until the specimen is completely dispersed. Extract with 25 mL of dimethylformamide, collecting the extract in a 50-mL volumetric flask. Repeat the extraction with two 10-mL portions of dimethylformamide, collecting the extracts in the 50-mL volumetric flask, and dilute with dimethylformamide to volume. Transfer 1.0 mL of this solution to a suitable size screw-capped vial, and evaporate the solution with the aid of nitrogen at 60° to dryness. Dissolve the residue in 1.0 mL of a mixture of pyridine and hexane (4:1), and pipet 1.0 mL of *N,O*-bis(trimethylsilyl)acetamide and 1.0 mL of *Internal standard solution* into the glass vial, fitted with a polytetrafluoroethylene septum, and securely close. Heat the vial on a water bath at 50° for 15 min, and cool to room temperature.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 2-mm × 1.8-m; packed with 3% liquid phase G3 on 80- to 100-mesh support SIAB

Temperatures

Column: 165°

Injection port: 170°

Detector: 250°

Carrier gas: Dry helium

Flow rate: 30 mL/min

Injection volume: 1 µL

#### System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for clioquinol and pyrene are 0.6 and 1.0, respectively.]

#### Suitability requirements

Resolution: NLT 3.0 between the analyte and internal standard peaks

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms to obtain NLT 40% of maximum recorder response, and measure the peak response of each component.

Calculate the percentage of the labeled amount of clioquinol (C<sub>9</sub>H<sub>5</sub>ClINO) taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of clioquinol to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of clioquinol to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Clioquinol RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clioquinol in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### • HYDROCORTISONE

Mobile phase: Acetonitrile, methanol, and water (1:1:2.75)

Standard stock solution: 1 mg/mL of USP Hydrocortisone RS in alcohol

Standard solution: *Standard stock solution* and alcohol (1:9)

Sample solution: Transfer nominally 10 mg of hydrocortisone from Ointment to a 50-mL centrifuge tube. Add 30 mL of alcohol, and heat on a steam bath just to boiling. Shake for 15 min, and centrifuge. Transfer the supernatant extract to a 100-mL volumetric flask. Repeat the extraction with two 20-mL portions of alcohol, combining the extracts in the 100-mL volumetric flask. Add alcohol to volume, mix, and filter.

System suitability stock solution: 0.5 mg/mL of methylparaben in alcohol

System suitability solution: Transfer 2 mL of *System suitability stock solution* and 20 mL of *Standard stock so-*

*lution* into a 200-mL volumetric flask, and dilute with alcohol to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Columns

Guard: Packing L2

Analytical: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

#### System suitability

Sample: *System suitability solution*

[NOTE—The relative retention times for methylparaben and hydrocortisone are 0.6 and 1.0, respectively.]

#### Suitability requirements

Resolution: NLT 2.0 between the hydrocortisone and methylparaben peaks

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of hydrocortisone (C<sub>21</sub>H<sub>30</sub>O<sub>5</sub>) taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Hydrocortisone RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of hydrocortisone in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

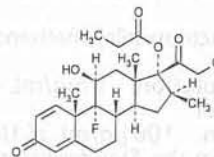
#### PERFORMANCE TESTS

- **MINIMUM FILL** (755): Meets the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)
  - USP Clioquinol RS
  - USP Hydrocortisone RS

## Clobetasol Propionate



C<sub>25</sub>H<sub>32</sub>ClFO<sub>5</sub> 466.97

Pregna-1,4-diene-3,20-dione, 21-chloro-9-fluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)-, (11β,16β)-; 21-Chloro-9-fluoro-11β,17-dihydroxy-16β-methylpregna-1,4-diene-3,20-dione 17-propionate [25122-46-7; 25122-41-2].

#### DEFINITION

Clobetasol Propionate contains NLT 97.0% and NMT 102.0% of C<sub>25</sub>H<sub>32</sub>ClFO<sub>5</sub>, calculated on the dried basis.

#### IDENTIFICATION

- **INFRARED ABSORPTION** (197M)

#### ASSAY

- **PROCEDURE**

**Solution A:** 0.05 M monobasic sodium phosphate. Adjust with 85% phosphoric acid to a pH of 2.5.



**Mobile phase:** Acetonitrile, methanol, and *Solution A* (19:4:17)

**Internal standard solution:** 0.2 mg/mL of beclomethasone dipropionate in methanol

**Standard solution:** Dissolve a quantity of USP Clobetasol Propionate RS in methanol and *Internal standard solution* to obtain a final solution of 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate.

**System suitability solution:** 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*

**Sample solution:** Transfer 4 mg of Clobetasol Propionate to a 100-mL volumetric flask, add 40.0 mL of *Internal standard solution*, and dilute with methanol to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A

**Column efficiency:** NLT 5000 theoretical plates for the clobetasol peak

**Tailing factor:** NMT 2.0 for the clobetasol peak

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]

Calculate the percentage of  $C_{25}H_{32}ClFO_5$  in the portion of Clobetasol Propionate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*

$R_S$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*

$C_S$  = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

#### IMPURITIES

##### Inorganic Impurities

- **RESIDUE ON IGNITION** (281): NMT 0.1%, using a platinum crucible

#### Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm (Official 1-Jan-2018)

##### Organic Impurities

##### • PROCEDURE

**Solution A**, **Mobile phase**, **System suitability solution**, and **Chromatographic system:** Proceed as directed in the *Assay*.

**Sample solution:** 0.1 mg/mL of Clobetasol Propionate in *Mobile phase*

#### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Clobetasol Propionate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak area for each impurity

$r_T$  = sum of the areas of all of the peaks

#### Acceptance criteria

**Any individual impurity:** NMT 1.0%

**Total impurities:** NMT 2.5%

#### SPECIFIC TESTS

- **MELTING RANGE OR TEMPERATURE** (741): Approximately 196°

- **OPTICAL ROTATION**, *Specific Rotation* (781S): +98° to +104° at 20°

**Sample solution:** 10 mg/mL in dioxane

- **LOSS ON DRYING** (731): Dry a sample at 105° for 3 h: it loses NMT 2.0% of its weight.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

- **USP REFERENCE STANDARDS** (11)

USP Clobetasol Propionate RS

USP Clobetasol Propionate Related Compound A RS

9α-Fluoro-11β-hydroxy-16β-methyl 3-oxo-androsta-1,4-diene-17(R)-spiro-2'-[4'-chloro-5'-ethylfuran-3'(2'H)-one].

$C_{25}H_{30}ClFO_4$  448.96

## Clobetasol Propionate Cream

#### DEFINITION

Clobetasol Propionate Cream is Clobetasol Propionate in a suitable cream base. It contains NLT 90.0% and NMT 115.0% of the labeled amount of clobetasol propionate ( $C_{25}H_{32}ClFO_5$ ).

#### IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Standard solution:** 0.6 mg/mL of USP Clobetasol Propionate RS in chloroform

**Test solution:** Transfer a portion of Cream equivalent to 0.75 mg of clobetasol propionate to a 25-mL, plastic-stoppered centrifuge tube. Add 10 mL of methanol, and cap. Heat in a 60° water bath for 4 min, remove the tube from the bath, and shake vigorously. Repeat the heating and shaking. Cool to room temperature, add 3.5 mL of water, and mix. Centrifuge at 3500 rpm for 10 min. Transfer 5 mL of the supernatant to a 100-mL separator, add 1 g of sodium chloride and 10 mL of water, and mix. Extract with 5 mL of chloroform by shaking for 1 min, collect the lower layer, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 0.5 mL of chloroform.

**Developing solvent system:** Chloroform, acetone, and alcohol (100:10:5)

**Acceptance criteria:** The  $R_f$  value of the principal spot obtained from the *Test solution* corresponds to that from the *Standard solution*.

#### ASSAY

##### • PROCEDURE

**Buffer:** 0.05 M monobasic sodium phosphate. Adjust with 50% sodium hydroxide solution to a pH of 5.5.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (95:20:85)



**Internal standard solution:** 0.2 mg/mL of beclomethasone dipropionate in methanol

**System suitability solution:** 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*

**Standard solution:** 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate prepared as follows. Transfer 1.0 mg of USP Clobetasol Propionate RS to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with methanol to volume.

**Sample solution:** Nominally 0.04 mg/mL of clobetasol propionate. In a suitable flask, dissolve a portion of Cream equivalent to 1.0 mg of clobetasol propionate in 10.0 mL of the *Internal standard solution* and 15.0 mL of methanol, and shake vigorously to disperse the Cream. Centrifuge at about 3500 rpm for 10 min, and pass a portion of the supernatant through a filter of 0.45- $\mu$ m pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 240 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection size:** 10  $\mu$ L

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A

**Column efficiency:** NLT 5000 theoretical plates for the clobetasol propionate peak

**Tailing factor:** NMT 2.0 for the clobetasol propionate peak

**Relative standard deviation:** NMT 2.0% for the clobetasol propionate peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]

Calculate the percentage of clobetasol propionate ( $C_{25}H_{32}ClFO_5$ ) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*

$R_S$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*

$C_S$  = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–115.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL** (755): Meets the requirements

#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed  $10^2$  cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.

- **PH** (791): 4.5–7.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers. Store at controlled room temperature. Do not refrigerate.
- **USP REFERENCE STANDARDS** (11)
  - USP Clobetasol Propionate RS
  - USP Clobetasol Propionate Related Compound A RS
  - 9 $\alpha$ -Fluoro-11 $\beta$ -hydroxy-16 $\beta$ -methyl 3-oxo-androsta-1,4-diene-17(R)-spiro-2'-[4'-chloro-5'-ethylfuran-3'(2'H)-one].
  - $C_{25}H_{30}ClFO_4$  448.96

## Clobetasol Propionate Ointment

#### DEFINITION

Clobetasol Propionate Ointment is Clobetasol Propionate in a suitable ointment base. It contains NLT 90.0% and NMT 115.0% of the labeled amount of clobetasol propionate ( $C_{25}H_{32}ClFO_5$ ).

#### IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Standard solution:** 0.5 mg/mL of of USP Clobetasol Propionate RS in chloroform

**Test solution:** Nominally 0.5 mg/mL of clobetasol propionate. Transfer a portion of Ointment equivalent to 1.0 mg of clobetasol propionate to a 25-mL, plastic-stoppered centrifuge tube. Add 10 mL of methanol, and cap. Heat in a 70° water bath for 4 min, remove the tube from the bath, and shake vigorously. Repeat the heating and shaking. Freeze the mixture in an ice bath for 5 min, and centrifuge at about 3500 rpm for 10 min. Transfer 5 mL of the supernatant to a suitable vial. Evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 1.0 mL of chloroform.

**Developing solvent system:** Chloroform, acetone, and alcohol (100:10:5)

**Acceptance criteria:** The  $R_f$  value of the principal spot obtained from the *Test solution* corresponds to that from the *Standard solution*.

#### ASSAY

- **PROCEDURE**

**Buffer:** 0.05 M monobasic sodium phosphate. Adjust with 85% phosphoric acid to a pH of 2.5.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (95:20:85)

**Internal standard solution:** 0.2 mg/mL of beclomethasone dipropionate in methanol

**System suitability solution:** 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase*

**Standard solution:** Dissolve a quantity of USP Clobetasol Propionate RS in methanol and *Internal standard solution* to obtain a final solution of 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate

**Sample solution:** Nominally 0.04 mg/mL of clobetasol propionate. Transfer a portion of Ointment equivalent to 1.0 mg of clobetasol propionate into a 125-mL separatory funnel. Add 30 mL of hexane, 10.0 mL of the *Internal standard solution*, and shake. Collect the lower layer in a 25-mL volumetric flask. Extract the hexane remaining in the separatory funnel with two 5-mL portions of *Mobile phase*, and combine all of the extracts in the 25-mL volumetric flask. Dilute with *Mobile phase* to volume, and pass a portion through a filter of 0.45- $\mu$ m pore size.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 240 nm**Column:** 4.6-mm × 15-cm; packing L1**Flow rate:** 1 mL/min**Injection size:** 10 µL**System suitability****Sample:** *System suitability solution*

[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

**Suitability requirements****Resolution:** NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A**Column efficiency:** NLT 5000 theoretical plates for the clobetasol propionate peak**Tailing factor:** NMT 2.0 for the clobetasol propionate peak**Relative standard deviation:** NMT 2.0% for the clobetasol propionate peak**Analysis****Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]

Calculate the percentage of clobetasol propionate (C<sub>25</sub>H<sub>32</sub>ClFO<sub>5</sub>) in the portion of Ointment taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*

$R_S$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*

$C_S$  = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–115.0%**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed 10<sup>2</sup> cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.
- **MINIMUM FILL** (755): Meets the requirements

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or in tight containers. Store at controlled room temperature. Do not refrigerate.
- **USP REFERENCE STANDARDS** (11)
  - USP Clobetasol Propionate RS
  - USP Clobetasol Propionate Related Compound A RS
  - 9α-Fluoro-11β-hydroxy-16β-methyl 3-oxo-androsta-1,4-diene-17(R)-spiro-2'-[4'-chloro-5'-ethylfuran-3'(2'H)-one].
  - C<sub>25</sub>H<sub>30</sub>ClFO<sub>4</sub> 448.96

**Clobetasol Propionate Topical Solution****DEFINITION**

Clobetasol Propionate Topical Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of clobetasol propionate (C<sub>25</sub>H<sub>32</sub>ClFO<sub>5</sub>).

**IDENTIFICATION**

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** (201)

**Standard solution:** 0.75 mg/mL of USP Clobetasol Propionate RS in chloroform**Test solution:** Nominally 0.75 mg/mL of clobetasol propionate. Transfer a portion of Topical Solution equivalent to 1.5 mg of clobetasol to a 50-mL separatory funnel. Add 5 mL of water, and extract with 5 mL of chloroform. Collect the lower layer through a cotton wool plug, and evaporate with the aid of a stream of nitrogen to dryness. Dissolve the residue in 2 mL of chloroform.**Developing solvent system:** Chloroform, acetone, and alcohol (100:10:5)**Acceptance criteria:** The  $R_f$  value of the principal spot obtained from the *Test solution* corresponds to that from the *Standard solution*.**ASSAY**

- **PROCEDURE**

**Buffer:** 0.05 M monobasic sodium phosphate. Adjust with 85% phosphoric acid to a pH of 2.5.**Mobile phase:** Acetonitrile, methanol, and *Buffer* (95:20:85)**Internal standard solution:** 0.2 mg/mL of beclomethasone dipropionate in methanol**System suitability solution:** 0.001 mg/mL of USP Clobetasol Propionate Related Compound A RS and 0.1 mg/mL of USP Clobetasol Propionate RS in *Mobile phase***Standard solution:** 0.04 mg/mL of USP Clobetasol Propionate RS and 0.08 mg/mL of beclomethasone dipropionate prepared as follows. Transfer 1.0 mg of USP Clobetasol Propionate RS to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with methanol to volume.**Sample solution:** Nominally 0.04 mg/mL of clobetasol propionate. Transfer a portion of Topical Solution equivalent to 1.0 mg of clobetasol propionate to a 25-mL volumetric flask, add 10.0 mL of the *Internal standard solution*, and dilute with *Mobile phase* to volume.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 240 nm**Column:** 4.6-mm × 15-cm; packing L1**Flow rate:** 1 mL/min**Injection size:** 10 µL**System suitability****Sample:** *System suitability solution*

[NOTE—The relative retention times for clobetasol propionate and clobetasol propionate related compound A are 1.0 and 1.1, respectively.]

**Suitability requirements****Resolution:** NLT 1.5 between clobetasol propionate and clobetasol propionate related compound A**Column efficiency:** NLT 5000 theoretical plates for the clobetasol propionate peak**Tailing factor:** NMT 2.0 for the clobetasol propionate peak**Relative standard deviation:** NMT 2.0% for the clobetasol propionate peak**Analysis****Samples:** *Standard solution* and *Sample solution*

[NOTE—The relative retention times for clobetasol propionate and beclomethasone dipropionate are 1.0 and 1.6, respectively.]

Calculate the percentage of clobetasol propionate (C<sub>25</sub>H<sub>32</sub>ClFO<sub>5</sub>) in the portion of Topical Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$



- $R_U$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Sample solution*
- $R_S$  = ratio of the clobetasol propionate peak area to the internal standard peak area from the *Standard solution*
- $C_S$  = concentration of USP Clobetasol Propionate RS in the *Standard solution* (mg/mL)
- $C_U$  = nominal concentration of clobetasol propionate in the *Sample solution* (mg/mL)
- Acceptance criteria: 90.0%–110.0%

**PERFORMANCE TESTS**

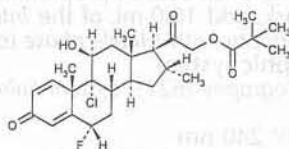
- **MINIMUM FILL** (755): Meets the requirements

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed  $10^2$  cfu/g. It meets the requirements of the tests for absence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species.
- **pH** (791): 4.5–6.0

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature. Do not refrigerate.
- **USP REFERENCE STANDARDS** (11)
  - USP Clobetasol Propionate RS
  - USP Clobetasol Propionate Related Compound A RS
  - 9 $\alpha$ -Fluoro-11 $\beta$ -hydroxy-16 $\beta$ -methyl 3-oxo-androsta-1,4-diene-17(R)-spiro-2'-[4'-chloro-5'-ethylfuran-3'(2'H)-one].
  - $C_{25}H_{30}ClFO_4$  448.96

**Clocortolone Pivalate**

$C_{27}H_{36}ClFO_5$  495.02

Pregna-1,4-diene-3,20-dione, 9-chloro-21-(2,2-dimethyl-1-oxopropoxy)-6-fluoro-11-hydroxy-16-methyl-, (6 $\alpha$ ,11 $\beta$ ,16 $\alpha$ )-.

9-Chloro-6 $\alpha$ -fluoro-11 $\beta$ ,21-dihydroxy-16 $\alpha$ -methylpregna-1,4-diene-3,20-dione 21-pivalate [34097-16-0].

» Clocortolone Pivalate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{27}H_{36}ClFO_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Clocortolone Pivalate RS

**Color and clarity of solution**—A 1 in 100 solution in chloroform is clear and practically colorless.

**Identification**—

A: **Infrared Absorption** (197M).

B: **Ultraviolet Absorption** (197U)—

*Solution:* 15  $\mu$ g per mL.

*Medium:* methanol.

Absorptivities at 238 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** (781S): between +125° and +135°.

*Test solution:* 40 mg per mL, in chloroform.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%, a 100-mg test specimen being used.

**Chromatographic purity**—

*Test solution*—Accurately weigh about 100 mg of Clocortolone Pivalate, and transfer to a 25-mL volumetric flask. Dissolve in a mixture of chloroform and methanol (1:1), and dilute with the same solvent to volume.

*Standard solution*—Using an accurately weighed quantity of USP Clocortolone Pivalate RS, prepare a solution in a mixture of chloroform and methanol (1:1) having a known concentration of about 4 mg per mL.

*Procedure*—Score a 20- × 20-cm thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture into three equal sections to be used for the *Test solution*, the blank, and the *Standard solution*, respectively. Activate the plate at 105° for 30 minutes before use. Apply 100  $\mu$ L each of the *Test solution* and the *Standard solution* as streaks 2.5 cm from the bottom of the appropriate section of the plate, and dry the streaks with a gentle current of air. Using a solvent system consisting of a mixture of cyclohexane and ethyl acetate (2:1), develop the chromatogram in a suitable chromatographic chamber lined with absorbent paper and previously equilibrated, until the solvent front has moved 15 cm above the line of application. Air-dry the plate, and develop the chromatogram a second time using the same chromatographic system. Air-dry the plate, and locate the principal band occupied by the *Standard solution* by viewing under UV light. Mark this band as well as corresponding bands in the blank and *Test solution* sections of the plate. Quantitatively remove the silica gel from each band, and transfer to separate glass-stoppered, 50-mL centrifuge tubes. Add 25.0 mL of methanol to each tube, shake for not less than 20 minutes, and centrifuge. Concomitantly determine the absorbances of the supernatants from the *Test solution* and the *Standard solution* against the blank at the wavelength of maximum absorbance at about 238 nm, with a suitable spectrophotometer. Calculate the percentage of chromatographic impurities in the *Test solution* taken by the formula:

$$100 - [100(C_S / C_U)(A_U / A_S)]$$

in which  $C_S$  is the concentration, in mg per mL, of USP Clocortolone Pivalate RS in the *Standard solution*;  $C_U$  is the concentration, in mg per mL, of the *Test solution*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Test solution* and the *Standard solution*, respectively: not more than 3.0% of total impurities is found.

**Assay**—

*Standard preparation*—Dissolve an accurately weighed quantity of USP Clocortolone Pivalate RS in chloroform to obtain a solution having a known concentration of about 0.75 mg per mL. Dilute an accurately measured volume of this solution with methanol, and mix to obtain a *Standard preparation* having a known concentration of about 30  $\mu$ g per mL.

*Assay preparation*—Accurately weigh about 75 mg of Clocortolone Pivalate, and transfer to a 100-mL volumetric flask. Dissolve in chloroform, dilute with chloroform to volume, and mix. Transfer 4.0 mL of this solution to a 100-mL volumetric flask, dilute with methanol to volume, and mix.

*Procedure*—Transfer 10.0-mL portions of the *Standard preparation* and the *Assay preparation* to separate glass-stoppered, 50-mL conical flasks, and evaporate on a steam bath to dryness. To each flask, and to a third flask to provide the blank, add 15.0 mL of a solution containing 250 mg of isoniazid and 0.3 mL of hydrochloric acid in 500 mL of metha-



nol. Swirl the contents of the flasks to dissolve the residues. Insert the stoppers securely in the flasks, and place in a water bath at 60° for 2.5 hours. Cool to room temperature. Concomitantly determine the absorbances of the *Assay preparation* and the *Standard preparation* in 1-cm cells against the blank at the wavelength of maximum absorbance at about 405 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of  $C_{27}H_{36}ClFO_5$  in the portion of Clo cortolone Pivalate taken by the formula:

$$2.5C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Clo cortolone Pivalate RS in the *Standard preparation*, and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

### Clocortolone Pivalate Cream

» Clo cortolone Pivalate Cream contains not less than 90.0 percent and not more than 110.0 percent of  $C_{27}H_{36}ClFO_5$  in a suitable cream base. It may contain suitable preservatives.

**Packaging and storage**—Preserve in collapsible tubes or in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Clo cortolone Pivalate RS

**Identification**—Place a portion of Cream, equivalent to about 1 mg of clo cortolone pivalate, in a suitable separator. Add 5 mL of water, and extract with 10 mL of chloroform. Evaporate the chloroform layer to dryness, and dissolve the residue in 2 mL of methanol. Apply 20  $\mu\text{L}$  of this test solution and 20  $\mu\text{L}$  of a Standard solution of USP Clo cortolone Pivalate RS in chloroform containing about 0.5 mg per mL about 1.5 cm from the bottom of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of cyclohexane and ethyl acetate (2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Minimum fill** (755): meets the requirements.

**pH** (791): between 5.0 and 7.0, in a 1 in 10 aqueous dispersion.

**Particle size determination**—Place a small portion of Cream on a microscope slide, apply a cover slide, press slightly, and examine under 40 $\times$  objective magnification using a suitable microscope equipped with polarized light. Scan the complete slide preparation, and record the size of the largest crystal found in reference to a calibrated grid: no particle in the Cream is greater than 50 microns when measured in the longitudinal axis.

#### Assay—

**Standard preparation**—Dissolve an accurately weighed quantity of USP Clo cortolone Pivalate RS in methanol to obtain a solution having a known concentration of about 0.06 mg per mL.

**Assay preparation**—Using a plastic syringe equipped with a suitable cannula, transfer an accurately weighed quantity of Cream, equivalent to about 3 mg of clo cortolone pivalate, to a 50-mL volumetric flask. Add about 25 mL of methanol, and warm the flask in a 60° water bath for about

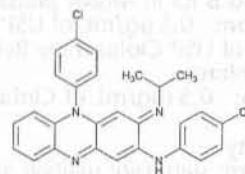
10 minutes, with occasional swirling, to disperse the Cream. Cool to room temperature, dilute with methanol to volume, and mix. Allow any insoluble material to settle.

**Procedure**—Transfer 10.0 mL of the *Standard preparation* to a 25-mL volumetric flask. Transfer 10.0-mL portions of the *Assay preparation* into two separate 25-mL volumetric flasks labeled *Assay preparation* and *Assay blank*, respectively. Evaporate the contents of the three flasks with the aid of a stream of air or nitrogen to dryness. Transfer 20.0 mL of a solution containing 250 mg of isoniazid and 0.3 mL of hydrochloric acid in 500 mL of methanol to the flasks containing the *Standard preparation*, the *Assay preparation*, and a fourth 25-mL volumetric flask labeled *Reagent blank*. Pipet 20 mL of acidified methanol solution (0.3 mL of hydrochloric acid diluted with methanol to 500 mL) into the flask labeled *Assay blank*. Insert the stoppers securely in the flasks, and place in a water bath at 60° for 2.5 hours, occasionally swirling the contents of each flask. Cool the flasks to room temperature, dilute with the acidified methanol solution to volume, and mix. Centrifuge the *Assay preparation* at high speed for 10 minutes. Concomitantly determine the absorbances of the *Standard preparation*, the *Assay preparation*, the *Assay blank*, and the *Reagent blank* against acidified methanol solution as the solvent blank in 1-cm cells at the wavelength of maximum absorbance at about 390 nm, with a suitable spectrophotometer. Calculate the quantity, in mg, of  $C_{27}H_{36}ClFO_5$  in the portion of Cream taken by formula:

$$50C(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Clo cortolone Pivalate RS in the *Standard preparation*;  $A_U$  is the absorbance of the *Assay preparation*, corrected for the *Assay blank* and the *Reagent blank*; and  $A_S$  is the absorbance of the *Standard preparation* corrected for the *Reagent blank*.

### Clofazimine



$C_{27}H_{22}Cl_2N_4$  473.40  
2-Phenazinamine, N,5-bis(4-chlorophenyl)-3,5-dihydro-3-[(1-methylethyl)imino]-;  
3-(p-Chloroanilino)-10-(p-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine [2030-63-9].

#### DEFINITION

Clofazimine contains NLT 98.0% and NMT 102.0% of clofazimine ( $C_{27}H_{22}Cl_2N_4$ ), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197): Use (197A) or (197K).
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Buffer:** 4.5 mg/mL of sodium dodecyl sulfate, 1.7 mg/mL of tetrabutylammonium hydrogen sulfate, and 1.8 mg/mL of disodium hydrogen phosphate in water. Adjust with dilute phosphoric acid (about 8.5%) to a pH of 3.0 in 90% of the volume before diluting with water to volume.



**Mobile phase:** Acetonitrile and Buffer (65:35)

**Standard solution:** 0.05 mg/mL of USP Clofazimine RS in *Mobile phase*

**Sample solution:** 0.05 mg/mL of Clofazimine in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5 for the clofazimine peak, *Standard solution*

**Relative standard deviation:** NMT 0.73% for the clofazimine peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clofazimine ( $C_{27}H_{22}Cl_2N_4$ ) in the portion of Clofazimine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Clofazimine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clofazimine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

• **RESIDUE ON IGNITION** (281): NMT 0.1%

• **ORGANIC IMPURITIES**

**Buffer, Mobile phase, and Chromatographic system:** Proceed as directed in the *Assay*.

**System suitability solution:** 0.5 mg/mL of USP

Clofazimine RS and 1.5 μg/mL of USP Clofazimine Related Compound B RS in *Mobile phase*

**Standard solution:** 0.5 μg/mL of USP Clofazimine RS and 5.0 μg/mL of USP Clofazimine Related Compound B RS in *Mobile phase*

**Sample solution:** 0.5 mg/mL of Clofazimine in *Mobile phase*

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between the clofazimine and clofazimine related compound B peaks, *System suitability solution*

**Relative standard deviation:** NMT 2.8% for the clofazimine peak and NMT 2.0% for the clofazimine related compound B peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clofazimine related compound B in the portion of Clofazimine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clofazimine related compound B from the *Sample solution*

$r_S$  = peak response of clofazimine related compound B from the *Standard solution*

$C_S$  = concentration of USP Clofazimine Related Compound B RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clofazimine in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified impurity in the portion of Clofazimine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any individual unspecified impurity from the *Sample solution*

$r_S$  = peak response of clofazimine from the *Standard solution*

$C_S$  = concentration of USP Clofazimine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clofazimine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 1. Disregard any impurity peaks less than 0.05%.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Clofazimine related compound B	0.81	1.0
Clofazimine	1.00	—
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

**SPECIFIC TESTS**

• **LOSS ON DRYING** (731)

**Analysis:** Dry a sample at 105° for 3 h.

**Acceptance criteria:** NMT 0.5%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers at room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Clofazimine RS

USP Clofazimine Related Compound B RS

5-(4-Chlorophenyl)-3-(isopropylimino)-N-phenyl-3,5-dihydrophenazin-2-amine.

$C_{27}H_{23}ClN_4$  438.95

**Clofazimine Capsules**

**DEFINITION**

Clofazimine Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of clofazimine ( $C_{27}H_{22}Cl_2N_4$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

• **PROCEDURE**

**Buffer:** 4.5 mg/mL of sodium dodecyl sulfate, 1.7 mg/mL of tetrabutylammonium hydrogen sulfate, and 1.8 mg/mL of disodium hydrogen phosphate in water. Adjust with dilute phosphoric acid (about 8.5%) to a pH of 3.0 in 90% of the volume before diluting with water to volume.

**Mobile phase:** Acetonitrile and Buffer (65:35)

**System suitability solution:** 0.5 mg/mL of USP

Clofazimine RS and 1.5 μg/mL of USP Clofazimine Related Compound B RS in *Mobile phase*

**Standard solution:** 0.05 mg/mL of USP Clofazimine RS in *Mobile phase*



**Sample stock solution:** Nominally 0.5 mg/mL of clofazimine in *Mobile phase* prepared as follows. Remove as completely as possible the contents of NLT 20 Capsules, and mix. Transfer the weighed portion of the combined contents of the Capsules, equivalent to about 500 mg of clofazimine, into a 250-mL conical flask. Add 50 mL of *Mobile phase* in increments, shake well, and quantitatively transfer into a 1000-mL volumetric flask. Repeat this process until transfer of all the Capsule contents is complete and make up the volume of the flask with *Mobile phase*. Stir at a high speed to make the solution homogenous.

**Sample solution:** Nominally 0.05 mg/mL of clofazimine from the *Sample stock solution* in *Mobile phase* prepared as follows. Filter 20 mL of the *Sample stock solution* into a beaker. Transfer 1.0 mL of the filtered solution into a 10-mL volumetric flask, and dilute to volume with *Mobile phase*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm. For *Identification test B*, use a diode array detector in the range of 190 nm–400 nm.

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Flow rate:** 1.0 mL/min

**Injection volume:** 20 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between clofazimine and clofazimine related compound B peaks, *System suitability solution*

**Tailing factor:** NMT 1.5 for the clofazimine peak, *Standard solution*

**Relative standard deviation:** NMT 1.0% for the clofazimine peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of clofazimine ( $C_{27}H_{22}Cl_2N_4$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clofazimine from the *Sample solution*

$r_S$  = peak response of clofazimine from the *Standard solution*

$C_S$  = concentration of USP Clofazimine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clofazimine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** Water; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 15 min

**Analysis:** Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting the rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

**Tolerances:** The requirements are met if all of the Capsules tested rupture in NMT 15 min. If 1 or 2 of the Capsules rupture in more than 15 but NMT 30 min, repeat the test on 12 additional Capsules. NMT 2 of the total of 18 Capsules tested rupture in more than 15 min but NMT 30 min.

##### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Buffer, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.5 μg/mL of USP Clofazimine RS and 5 μg/mL of USP Clofazimine Related Compound B RS in *Mobile phase*

**Sample solution:** Nominally 0.5 mg/mL of clofazimine in *Mobile phase* prepared as follows. Remove as completely as possible the contents of NLT 20 Capsules, and mix. Transfer the weighed portion of the combined contents of the Capsules, equivalent to about 500 mg of clofazimine, into a 250-mL conical flask. Add 50 mL of *Mobile phase* in increments, shake well, and quantitatively transfer into a 1000-mL volumetric flask. Repeat this process until transfer of all the Capsule contents is complete and make up the flask volume with *Mobile phase*. Stir at a high speed to make the solution homogenous and filter.

#### Suitability requirements

**Resolution:** NLT 2.0 between the clofazimine and clofazimine related compound B peaks, *System suitability solution*

**Relative standard deviation:** NMT 2.8% for the clofazimine peak, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clofazimine related compound B in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clofazimine related compound B from the *Sample solution*

$r_S$  = peak response of clofazimine related compound B from the *Standard solution*

$C_S$  = concentration of USP Clofazimine Related Compound B RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clofazimine in the *Sample solution* (mg/mL)

Calculate the percentage of unspecified impurities in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of impurities from the *Sample solution*

$r_S$  = peak response of clofazimine from the *Standard solution*

$C_S$  = concentration of USP Clofazimine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clofazimine in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 1*. Disregard any impurity peaks less than 0.05%.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Clofazimine related compound B	0.81	1.0
Clofazimine	1.00	—
Any individual unspecified impurity	—	0.10
Total impurities	—	2.0

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.



• **USP REFERENCE STANDARDS** (11)

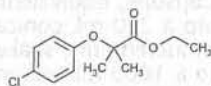
USP Clofazimine RS

USP Clofazimine Related Compound B RS

5-(4-Chlorophenyl)-3-(isopropylimino)-N-phenyl-3,5-dihydrophenazin-2-amine.

C<sub>27</sub>H<sub>23</sub>ClN<sub>4</sub> 438.95

## Clofibrate



C<sub>12</sub>H<sub>15</sub>ClO<sub>3</sub> 242.70

Propanoic acid, 2-(4-chlorophenoxy)-2-methyl-, ethyl ester.

Ethyl 2-(p-chlorophenoxy)-2-methylpropanoate [637-07-0].

» Clofibrate contains not less than 97.0 percent and not more than 103.0 percent of C<sub>12</sub>H<sub>15</sub>ClO<sub>3</sub>, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Clofibrate RS

**Identification**—

A: Infrared Absorption (197F).

B: Ultraviolet Absorption (197U)—

Solution: 20 µg per mL.

Medium: methanol.

**Refractive index** (831): between 1.500 and 1.505, at 20°.

**Acidity**—Mix 10.0 g with 100 mL of neutralized alcohol, add 3 drops of phenolphthalein TS, and titrate with 0.10 N sodium hydroxide: not more than 0.90 mL is required for neutralization.

**Water Determination, Method I** (921): not more than 0.2%.

**Chromatographic purity**—

**Standard preparation**—Prepare a solution in chloroform having known concentrations of about 0.1 mg of USP Clofibrate RS and 0.03 mg of p-chlorophenol per mL. To 10.0 mL of this solution add 5.0 µL of tributyrin, and mix.

**Test preparation**—To 10.0 mL of Clofibrate add 5.0 µL of tributyrin, and mix.

**Chromatographic system** (see *Chromatography* (621))—The gas chromatograph is equipped with a split injector with a 20:1 split ratio, a flame-ionization detector, and a 0.53-mm × 15-m column to the internal walls of which is bonded a 1.5-µm film of phase G1. The chromatograph is programmed to maintain the column temperature at 120° for 1 minute, then to increase the temperature at a rate of 5° per minute for 12 minutes, and finally to maintain a temperature of 180° for 9 minutes. Maintain the injection port at 210° and the detector block at 220°. Helium is used as the carrier gas flowing at a rate of about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative retention times are about 0.2 for p-chlorophenol, 0.55 for clofibrate, and 1.0 for tributyrin.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 1 µL) of the *Standard preparation* and the *Test preparation* into the

chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity, other than p-chlorophenol, in the Clofibrate taken by the formula:

$$0.1C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of USP Clofibrate RS in the *Standard preparation*,  $R_U$  is the ratio of the response of each individual impurity peak (other than the p-chlorophenol peak) to that of the tributyrin peak obtained from the *Test preparation*, and  $R_S$  is the ratio of the response of the clofibrate peak to that of the tributyrin peak obtained from the *Standard preparation*: the percentage of any impurity, other than p-chlorophenol, does not exceed 0.01%, and the total percentage of all such impurities does not exceed 0.12%.

**Limit of p-chlorophenol**—Use the chromatograms obtained in the test for *Chromatographic purity*. Calculate the percentage of p-chlorophenol in the portion of Clofibrate taken by the formula:

$$0.1C(R_U / R_S)$$

in which C is the concentration, in mg per mL, of p-chlorophenol in the *Standard preparation*, and  $R_U$  and  $R_S$  are the ratios of the responses of the p-chlorophenol and tributyrin peaks obtained from the *Test preparation* and the *Standard preparation*, respectively: not more than 0.003% of p-chlorophenol is found.

**Assay**—

**Ion-exchange resin**—To a beaker containing 75 mL of 1 N sodium hydroxide add about 3 g of a 50- to 100-mesh strongly basic styrene-divinylbenzene anion-exchange resin, and allow the mixture to stand for about 15 minutes, with occasional stirring. Wash the resin with water until the last washing is neutral to litmus paper, then wash with three 50-mL portions of methanol.

**Ion-exchange column**—Place a plug of glass wool in the base of a 1- × 15-cm ion-exchange tube, and transfer to the tube a sufficient amount of *ion-exchange resin*, slurried in methanol, to produce a column bed height of from 6 cm to 8 cm.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Clofibrate RS in methanol, and dilute quantitatively and stepwise with methanol to obtain a solution having a known concentration of about 20 µg per mL.

**Assay preparation**—Transfer about 200 mg of Clofibrate, accurately weighed, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 10.0 mL of this solution to the *ion-exchange column*, and collect the eluate in a 100-mL volumetric flask. Rinse the column with 25 mL of methanol, collect the rinsing in the volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 226 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of C<sub>12</sub>H<sub>15</sub>ClO<sub>3</sub> in the portion of Clofibrate taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Clofibrate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.



## Clofibrate Capsules

» Clofibrate Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{12}H_{15}ClO_3$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

### USP Reference standards (11)—

USP Clofibrate RS

**Identification**—Capsules respond to *Identification test A* under *Clofibrate*.

### Dissolution (711)—

**Medium:** sodium lauryl sulfate solution (5 in 100); 1000 mL.

**Apparatus 2:** 100 rpm.

**Time:** 180 minutes.

Determine the amount of  $C_{12}H_{15}ClO_3$  dissolved by employing the following method.

**Mobile phase**—Prepare a degassed and filtered mixture of methanol and water (80:20).

**Standard solution**—Transfer about 20 mg of USP Clofibrate RS, accurately weighed, to a 50-mL volumetric flask. Add 20 mL of methanol, mix to dissolve the clofibrate, dilute with water to volume, and mix. Dilute an aliquot quantitatively with methanol to obtain a final solution having a known concentration of about 80 µg per mL.

**Test solution**—Dilute a 5.0-mL portion of the solution under test to 25.0 mL, using methanol. Allow to stand for 5 minutes, and filter.

**Chromatographic system**—The liquid chromatograph is equipped with a 226-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal portions (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the amount of  $C_{12}H_{15}ClO_3$  dissolved.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{12}H_{15}ClO_3$  is dissolved in 180 minutes.

**Uniformity of dosage units** (905): meet the requirements.

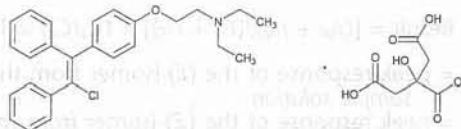
**Assay**—Proceed with Capsules as directed in the *Assay* under *Clofibrate*, using the following as the *Assay preparation*: Weigh accurately not less than 20 Capsules in a tared weighing bottle. With a sharp blade, carefully open the Capsules, without loss of shell material, and transfer the contents to a 100-mL beaker. Remove any liquid from the emptied capsules by washing with several small portions of ether. Discard the washings, and allow the capsules to dry in a jet of dry air until the odor of ether no longer is perceptible. Weigh the empty capsules in the tared weighing bottle, and calculate the average net weight per capsule. Transfer an accurately weighed amount of capsule contents, equivalent to about 200 mg of clofibrate, to a 100-mL volumetric flask, add methanol to volume, and mix. Transfer 10.0 mL of this solution to the *Ion-exchange column*, and collect the eluate in a 100-mL volumetric flask. Rinse the column with 25 mL of methanol, collect the rinsings in the volumetric flask, dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Calculate the

quantity, in mg, of  $C_{12}H_{15}ClO_3$  in the portion of Capsules taken by the formula:

$$10C(A_U / A_S)$$

in which C is the concentration, in µg per mL, of USP Clofibrate RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Clomiphene Citrate



$C_{26}H_{28}ClNO \cdot C_6H_8O_7$  598.08  
 Ethanamine, 2-[4-(2-chloro-1,2-diphenylethenyl)phenoxy]-N,N-diethyl-, 2-hydroxy-1,2,3-propanetricarboxylate (1:1);  
 2-[p-(2-Chloro-1,2-diphenylvinyl)phenoxy]triethylamine citrate (1:1) [50-41-9].

### DEFINITION

Clomiphene Citrate contains NLT 98.0% and NMT 102.0% of a mixture of the (E)- and (Z)-geometric isomers of clomiphene citrate ( $C_{26}H_{28}ClNO \cdot C_6H_8O_7$ ), calculated on the anhydrous basis. It contains NLT 30.0% and NMT 50.0% of the Z-isomer, [(Z)-2-[4-(2-chloro-1,2-diphenylethenyl)phenoxy]-N,N-diethylethanamine 2-hydroxy-1,2,3-propanetricarboxylate (1:1)].

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)  
**Medium:** 0.1 N hydrochloric acid  
**Sample solution:** 20 µg/mL  
**Acceptance criteria:** Meets the requirements
- **C. IDENTIFICATION TESTS—GENERAL, Citrate** (191)

### ASSAY

- **PROCEDURE**  
 Use low-actinic glassware for all solutions.  
**Mobile phase:** Methanol, water, and triethylamine (55: 45: 0.3). Adjust with phosphoric acid to a pH of 2.5.  
**System suitability solution:** 2 µg/mL of USP Clomiphene Related Compound A RS and 50 µg/mL of USP Clomiphene Citrate RS, in *Mobile phase*  
**Standard solution:** 50 µg/mL of USP Clomiphene Citrate RS, in *Mobile phase*  
**Sample stock solution:** 0.5 mg/mL of Clomiphene Citrate, in *Mobile phase*, filtered  
**Sample solution:** 50 µg/mL of Clomiphene Citrate, in *Mobile phase*, from *Sample stock solution*  
**Chromatographic system**  
 (See *Chromatography* (621), *System Suitability*.)  
**Mode:** LC  
**Detector:** UV 233 nm  
**Column:** 4.6-mm × 25-cm; packing L26  
**Flow rate:** 1 mL/min  
**Injection volume:** 50 µL  
**System suitability**  
**Samples:** *System suitability solution* and *Standard solution*  
 [NOTE—The relative retention times for clomiphene related compound A, (Z)-isomer, and (E)-isomer are about 0.9, 1.0, and 1.2, respectively.]



**Suitability requirements**

**Resolution:** NLT 1.0 between clomiphene related compound A and (Z)-isomer; NLT 1.5 between (Z)-isomer and (E)-isomer, *System suitability solution*

**Column efficiency:** NLT 2000 theoretical plates for the (E)-isomer, *Standard solution*

**Tailing factor:** NMT 3.0 for the (E)-isomer, *Standard solution*

**Relative standard deviation:** NMT 2.0% for both (E)- and (Z)-isomers, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clomiphene citrate ( $C_{26}H_{28}ClNO \cdot C_6H_8O_7$ ) in the portion of Clomiphene Citrate taken:

$$\text{Result} = [(r_{UE} + r_{UZ}) / (r_{SE} + r_{SZ})] \times (C_S / C_U) \times 100$$

$r_{UE}$  = peak response of the (E)-isomer from the *Sample solution*

$r_{UZ}$  = peak response of the (Z)-isomer from the *Sample solution*

$r_{SE}$  = peak response of the (E)-isomer from the *Standard solution*

$r_{SZ}$  = peak response of the (Z)-isomer from the *Standard solution*

$C_S$  = concentration of USP Clomiphene Citrate RS in the *Standard solution* (μg/mL)

$C_U$  = concentration of Clomiphene Citrate in the *Sample solution* (μg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous basis

**OTHER COMPONENTS**• **CONTENT OF (Z)-ISOMER**

**Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay.

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of (Z)-isomer in the portion of Clomiphene Citrate taken:

$$\text{Result} = (r_Z / r_T) \times 100$$

$r_Z$  = peak response of the (Z)-isomer from the *Sample solution*

$r_T$  = sum of all the peak responses of the *Sample solution*

**Acceptance criteria:** 30.0%–50.0%

**IMPURITIES****Delete the following:**

• **HEAVY METALS, Method II (231):** NMT 20 ppm • (Official 1-Jan-2018)

• **ORGANIC IMPURITIES**

Use low-actinic glassware for all solutions.

**Buffer:** Acetonitrile, diethylamine, and water (40: 0.8: 60). Adjust with phosphoric acid to a pH of 6.2.

**Solution A:** *Buffer* and water (90:10)

**Solution B:** *Buffer*

**Mobile phase:** See Table 1.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3.0	100	0
23.0	0	100
33.0	0	100
33.5	100	0
40.0	100	0

**System suitability stock solution:** 0.28 mg/mL of USP Clomiphene Related Compound A RS in *Buffer*

**System suitability solution:** 1.25 mg/mL of USP Clomiphene Citrate RS and 0.028 mg/mL of USP Clomiphene Related Compound A RS, prepared as follows. Transfer 12.5 mg of USP Clomiphene Citrate RS into a 10-mL volumetric flask, add 1.0 mL of *System suitability stock solution*, and dilute with *Buffer* to volume.

**Standard solution:** 0.025 mg/mL of USP Clomiphene Citrate RS in *Buffer*

**Sample solution:** 1.25 mg/mL of Clomiphene Citrate in *Buffer*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 233 nm

**Column:** 4.6-mm × 10-cm; 2.6-μm packing L7

**Column temperature:** 30°

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See Table 2 for relative retention times.]

**Suitability requirements**

**Relative standard deviation:** NMT 5.0% from the sum of the peak areas of the (E)- and (Z)-isomers, *Standard solution*

**Peak-to-valley ratio:** The ratio of the height of the clomiphene related compound A peak to the height of the valley between clomiphene related compound A and clomiphene peaks is NLT 15, *System suitability solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Clomiphene Citrate taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = sum of the peak responses of (E)- and (Z)-isomers of clomiphene from the *Standard solution*

$C_S$  = concentration of USP Clomiphene Citrate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clomiphene Citrate in the *Sample solution* (mg/mL)

$F$  = relative response factor (see Table 2)

**Acceptance criteria:** See Table 2. Disregard peaks less than 0.05%.



Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Clomiphene benzophenone analog <sup>a</sup>	0.10	0.51	1.0
Clomiphene tritylphenone analog <sup>b</sup>	0.13	1.0	1.0
Clomiphene keto analog <sup>c</sup>	0.33	1.0	1.0
Clomiphene related compound A	0.92	1.0	2.0
Clomiphene Z-isomer	0.98	—	—
Clomiphene E-isomer	1.00	—	—
2-Chloroclomiphene <sup>d</sup>	1.57	1.0	1.0 <sup>e</sup>
2-Chloroclomiphene <sup>d</sup>	1.63	1.0	
4-Chloroclomiphene <sup>d</sup>	1.70	1.0	
4-Chloroclomiphene <sup>d</sup>	1.77	1.0	
Deschloroclomiphene chlorophenyl analog <sup>g</sup>	2.36	0.77	1.0 <sup>e</sup>
Deschloroclomiphene chlorophenyl analog <sup>g</sup>	2.48	0.77	
Benzyl clomiphene <sup>h</sup>	2.67	0.74	0.15
Benzyl clomiphene <sup>h</sup>	2.76	0.81	0.15
Any other unspecified impurity	—	1.0	0.10
Total impurities	—	—	2.5

<sup>a</sup> 4-[2-(Diethylamino)ethoxy]phenyl(phenyl)methanone.<sup>b</sup> 2,2-Bis[4-[2-(diethylamino)ethoxy]phenyl]-1,2-diphenylethanone.<sup>c</sup> 2-[4-[2-(Diethylamino)ethoxy]phenyl]-1,2-diphenylethan-1-one.<sup>d</sup> (E,Z)-2-[2-Chloro-4-(2-chloro-1,2-diphenylvinyl)phenoxy]-N,N-diethylethan-1-amine.<sup>e</sup> The sum of these geometric isomers is NMT 1.0%.<sup>f</sup> (E,Z)-2-[4-[2-Chloro-2-(4-chlorophenyl)-1-phenylvinyl]phenoxy]-N,N-diethylethan-1-amine.<sup>g</sup> (E,Z)-2-[4-[1,2-Bis(4-chlorophenyl)vinyl]phenoxy]-N,N-diethylethan-1-amine.<sup>h</sup> (E,Z)-2-[4-[1-(4-Benzylphenyl)-2-chloro-2-phenylvinyl]phenoxy]-N,N-diethylethan-1-amine.**SPECIFIC TESTS**

- **WATER DETERMINATION, Method I (921):** NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**
  - USP Clomiphene Citrate RS
  - USP Clomiphene Related Compound A RS
  - (E,Z)-2-[4-(1,2-Diphenylethyl)phenoxy]-N,N-diethylethanamine hydrochloride.
  - C<sub>26</sub>H<sub>29</sub>NO · HCl 407.98

**Clomiphene Citrate Tablets****DEFINITION**

Clomiphene Citrate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of clomiphene citrate (C<sub>26</sub>H<sub>28</sub>ClNO · C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>).

**IDENTIFICATION**

- **A. IDENTIFICATION—ORGANIC NITROGENOUS BASES (181)**

**Sample solution:** Place a portion of finely powdered Tablets equivalent to about 30 mg of clomiphene citrate in a centrifuge tube containing a 30-mL solution of methanol and 0.1 N hydrochloric acid (1:2). Insert the stopper, and place the tube in a water bath at about 37° for 15 min. Shake occasionally. Centrifuge, and

place the clear supernatant in a separator. Extract with one 40-mL and two 25-mL portions of hexanes, and discard the extract. Render the aqueous solution alkaline with 1 N sodium hydroxide, and extract the precipitated base with one 50-mL and two 25-mL portions of hexanes. Wash the combined extracts with two portions of water. Dry the extract with anhydrous sodium sulfate, and remove the hexanes by evaporation under reduced pressure. Add about 1.0 mL of carbon disulfide to the residue, and dissolve.

**Standard solution:** Prepare as directed in the *Sample solution*, but use USP Clomiphene Citrate RS.

**Analysis**

**Samples:** *Sample solution* and *Standard solution*  
Determine the absorption spectra.

**Acceptance criteria:** Meet the requirements

**ASSAY**• **ANALYSIS**

**Mobile phase:** Methanol, water, and triethylamine (55: 45: 0.3). Adjust with phosphoric acid to a pH of 2.5.

**System suitability solution:** 0.002 mg/mL of USP Clomiphene Related Compound A RS and 0.05 mg/mL of USP Clomiphene Citrate RS in *Mobile phase*. Use actinic glassware for the *Standard solution* and *Sample solution*.

**Standard solution:** 0.05 mg/mL of USP Clomiphene Citrate RS, in *Mobile phase*

**Sample stock solution:** Nominally 0.5 mg/mL of clomiphene citrate prepared in *Mobile phase* as follows. Transfer an equivalent of 50 mg of clomiphene citrate from NLT 20 finely powdered Tablets to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase*, and stir using a magnetic bar for 30 min. Remove the magnetic bar from the flask, dilute with *Mobile phase* to volume, and filter.

**Sample solution:** Nominally 0.05 mg/mL of clomiphene citrate prepared from the *Sample stock solution* in *Mobile phase*. Filter, and discard the first 10 mL. [NOTE—This solution is stable for NLT 24 h.]

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 233 nm

**Column:** 4.6-mm × 25-cm; packing L26

**Flow rate:** 1.0 mL/min

**Injection volume:** 50 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for clomiphene related compound A, (Z)-isomer, and (E)-isomer are about 0.9, 1.0, and 1.2, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.0 between clomiphene related compound A and (Z)-isomer, and NLT 1.5 between (Z)-isomer and (E)-isomer, *System suitability solution*

**Column efficiency:** NLT 2000 theoretical plates for the (E)-isomer, *Standard solution*

**Tailing factor:** NMT 3.0 for the (E)-isomer, *Standard solution*

**Relative standard deviation:** NMT 2.0% for both (E)- and (Z)-isomers, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of clomiphene citrate (C<sub>26</sub>H<sub>28</sub>ClNO · C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*



$C_S$  = concentration of USP Clomiphenes Citrate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clomiphenes citrate in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–107.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 1: 100 rpm

Time: 30 min

Standard solution: USP Clomiphenes Citrate RS in 0.1 N hydrochloric acid

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute with 0.1 N hydrochloric acid to a concentration similar to the *Standard solution*.

Instrumental conditions

Mode: UV

Analytical wavelength: 232 nm

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clomiphenes citrate ( $C_{26}H_{28}ClNO \cdot C_6H_8O_7$ ) dissolved.

Tolerances: NLT 75% (Q) of the labeled amount of clomiphenes citrate ( $C_{26}H_{28}ClNO \cdot C_6H_8O_7$ ) dissolved

#### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE:

Preserve in well-closed containers, protected from light.

#### • USP REFERENCE STANDARDS (11)

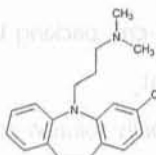
USP Clomiphenes Citrate RS

USP Clomiphenes Related Compound A RS

(E,Z)-2-[4-(1,2-diphenylethenyl)phenoxy]-N,N-diethylethanamine hydrochloride.

$C_{26}H_{29}NO \cdot HCl$  407.98

## Clomipramine Hydrochloride



$C_{19}H_{23}ClN_2 \cdot HCl$  351.31

5H-Dibenz[b,f]azepine-5-propanamine, 3-chloro-10,11-dihydro-N,N-dimethyl-, monohydrochloride;

3-Chloro-5-[3-(dimethylamino)propyl]-10,11-dihydro-5H-dibenz[b,f]azepine monohydrochloride [17321-77-6].

### DEFINITION

Clomipramine Hydrochloride contains NLT 98.0% and NMT 102.0% of clomipramine hydrochloride ( $C_{19}H_{23}ClN_2 \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

#### • B. ULTRAVIOLET ABSORPTION (197U)

Medium: 0.1 N hydrochloric acid

Solution: 100 µg/mL

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 1.0% at the wavelength of maximum absorbance.

### ASSAY

#### • PROCEDURE

**Solution A:** 55 g/L of sodium 1-heptanesulfonate prepared as follows. Transfer a suitable quantity of sodium 1-heptanesulfonate to an appropriate volumetric flask. Dissolve in 50% of the flask volume of water, and dilute with glacial acetic acid to volume.

**Mobile phase:** Transfer 20.0 mL of *Solution A* and 2.0 mL of triethylamine to a 500-mL volumetric flask, and dilute with water to volume. Transfer this solution to a 1-L volumetric flask, adjust with phosphoric acid to a pH of  $3.2 \pm 0.1$ , dilute with acetonitrile to volume, filter, and degas.

**System suitability solution:** 0.07 mg/mL of USP Desipramine Hydrochloride RS and 0.10 mg/mL of USP Imipramine Hydrochloride RS in methanol

**Standard solution:** 0.32 mg/mL of USP Clomipramine Hydrochloride RS in methanol

**Sample solution:** 0.32 mg/mL of Clomipramine Hydrochloride in methanol

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm  $\times$  30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 10 µL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for desipramine and imipramine are 0.85 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 0.5 between desipramine and imipramine

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clomipramine hydrochloride ( $C_{19}H_{23}ClN_2 \cdot HCl$ ) in the portion of Clomipramine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Clomipramine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clomipramine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

#### • RESIDUE ON IGNITION (281):

NMT 0.1%

#### Delete the following:

#### • HEAVY METALS, Method II (231):

NMT 100 ppm (Official 1-Jan-2018)

#### • ORGANIC IMPURITIES, PROCEDURE 1

**Solution A and System suitability solution:** Proceed as directed in the Assay.

**Mobile phase:** Transfer 20.0 mL of *Solution A*, 2.0 mL of triethylamine, and 500 mL of water to a suitable container. Adjust with phosphoric acid to a pH of  $3.2 \pm 0.1$ , and dilute with water to 625 mL. Transfer to a 1-L volumetric flask, and dilute with acetonitrile to volume.

**Sample solution:** 2 mg/mL of Clomipramine Hydrochloride in methanol

**Chromatographic system and System suitability:** Proceed as directed in the Assay, except use an *Injection volume* of 5 µL.



**Analysis****Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Clomipramine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak response of each impurity $r_T$  = sum of all the peak responses**Acceptance criteria:** See Table 1.• **ORGANIC IMPURITIES, PROCEDURE 2****Solution A, Mobile phase, System suitability solution, Chromatographic system, and System suitability:**Proceed as directed in the *Assay*.**Sample solution:** 2 mg/mL of Clomipramine Hydrochloride in methanol**Analysis****Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Clomipramine Hydrochloride taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 $r_U$  = peak response of each impurity $r_T$  = sum of the responses of all the peaks**Acceptance criteria:** See Table 1.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Any individual, unspecified impurity	—	0.5
Total impurities <sup>a</sup>	—	2.0

<sup>a</sup> Sum of all impurities from *Organic Impurities, Procedure 1* and *Organic Impurities, Procedure 2*.**SPECIFIC TESTS**• **pH (791)****Sample solution:** 100 mg/mL of Clomipramine Hydrochloride in water**Acceptance criteria:** 3.5–5.0• **Loss on Drying (731)****Analysis:** Dry at 105° for 2 h.**Acceptance criteria:** NMT 1.0%**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed containers.• **USP REFERENCE STANDARDS (11)**

USP Clomipramine Hydrochloride RS

USP Desipramine Hydrochloride RS

USP Imipramine Hydrochloride RS

**Clomipramine Hydrochloride Capsules****DEFINITION**Clomipramine Hydrochloride Capsules contain NLT 90.0% and NMT 110.0% of clomipramine hydrochloride ( $C_{19}H_{23}ClN_2 \cdot HCl$ ).**IDENTIFICATION**• **A. INFRARED ABSORPTION (197K)****Sample:** Transfer the contents of a number of Capsules, equivalent to about 125 mg of clomipramine hydrochloride, to a suitable container. Add 25 mL of chloroform, stir for 5 min, and filter. Evaporate on a steam bath to a volume of 5 mL, chill in an ice bath, add ethyl ether, and stir until crystals form. Filter, and dry at 100° for 1 h.**Acceptance criteria:** Meet the requirements**ASSAY**• **PROCEDURE****Solution A:** 55 g/L of sodium 1-heptanesulfonate prepared as follows. Transfer a suitable quantity of sodium 1-heptanesulfonate to an appropriate volumetric flask. Dissolve in 50% of the flask volume of water, and dilute with glacial acetic acid to volume.**Mobile phase:** Transfer 20.0 mL of *Solution A* and 2.0 mL of triethylamine to a 500-mL volumetric flask, and dilute with water to volume. Transfer this solution to a 1-L volumetric flask, adjust with phosphoric acid to a pH of  $3.2 \pm 0.1$ , dilute with acetonitrile to volume, filter, and degas.**System suitability solution:** 0.07 mg/mL of USP

Desipramine Hydrochloride RS and 0.10 mg/mL of USP Imipramine Hydrochloride RS in methanol

**Standard solution:** 0.32 mg/mL of USP Clomipramine Hydrochloride RS in methanol**Sample stock solution:** Nominally 0.8 mg/mL of clomipramine hydrochloride from the contents of NLT 20 Capsules in methanol prepared as follows. Transfer a suitable quantity of the contents of Capsules to an appropriate volumetric flask. Add 65% of the flask volume of methanol, shake by mechanical means for 1 h, and dilute with methanol to volume.**Sample solution:** Nominally 0.32 mg/mL of clomipramine hydrochloride from *Sample stock solution* in methanol prepared as follows. Transfer a suitable portion of *Sample stock solution* to an appropriate volumetric flask, dilute with methanol to volume, and filter. Use the filtrate.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 3.9-mm  $\times$  30-cm; packing L1**Flow rate:** 1 mL/min**Injection volume:** 10  $\mu$ L**System suitability****Sample:** *System suitability solution*

[NOTE—The relative retention times for desipramine and imipramine are 0.85 and 1.0, respectively.]

**Suitability requirements****Resolution:** NLT 0.5 between desipramine and imipramine**Relative standard deviation:** NMT 2.0%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of clomipramine hydrochloride ( $C_{19}H_{23}ClN_2 \cdot HCl$ ) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Clomipramine Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = nominal concentration of clomipramine hydrochloride in the *Sample solution* (mg/mL)**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**• **DISSOLUTION (711)****Medium:** 0.1 N hydrochloric acid; 500 mL**Apparatus 2:** 50 rpm**Time:** 30 min**Standard solution:** USP Clomipramine Hydrochloride RS in *Medium***Sample solution:** Sample per *Dissolution* (711), and filter the resulting solution. Dilute with *Medium*, if neces-



sary, to a concentration that is similar to the *Standard solution*.

#### Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 252 nm

#### Analysis

Samples: *Standard solution* and *Sample solution*

Determine the percentage of the labeled amount of clomipramine hydrochloride ( $C_{19}H_{23}ClN_2 \cdot HCl$ ) dissolved.

Tolerances: NLT 80% (Q) of the labeled amount of clomipramine hydrochloride ( $C_{19}H_{23}ClN_2 \cdot HCl$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905)

##### Procedure for content uniformity

Standard solution: 30 µg/mL of USP Clomipramine Hydrochloride RS in methanol

Sample stock solution: Transfer the contents of 1 Capsule to a 100-mL volumetric flask with the aid of methanol. Add about 75 mL of methanol, shake by mechanical means for 1 h, and dilute with methanol to volume.

Sample solution: Nominally 30 µg/mL of clomipramine hydrochloride from the *Sample stock solution* in methanol

#### Instrumental conditions

Mode: UV

Analytical wavelength: Maximum absorbance at about 252 nm

Cell: 1 cm

Blank: Methanol

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clomipramine hydrochloride ( $C_{19}H_{23}ClN_2 \cdot HCl$ ) in the portion of Capsules taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

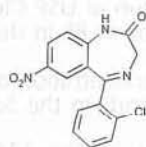
- $A_U$  = absorbance of the *Sample solution*  
 $A_S$  = absorbance of the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (µg/mL)  
 $C_U$  = nominal concentration of the *Sample solution* (µg/mL)

Acceptance criteria: Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
 USP Clomipramine Hydrochloride RS  
 USP Desipramine Hydrochloride RS  
 USP Imipramine Hydrochloride RS

## Clonazepam



$C_{15}H_{10}ClN_3O_3$  315.71

2H-1,4-Benzodiazepin-2-one, 5-(2-chlorophenyl)-1,3-dihydro-7-nitro-

5-(*o*-Chlorophenyl)-1,3-dihydro-7-nitro-2H-1,4-benzodiazepin-2-one [1622-61-3].

» Clonazepam contains not less than 98.0 percent and not more than 102.0 percent of  $C_{15}H_{10}ClN_3O_3$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers, at room temperature.

#### USP Reference standards (11)—

USP Clonazepam RS

USP Clonazepam Related Compound A RS

3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyryl.  
 $C_{15}H_{10}ClN_3O_3$  315.72

USP Clonazepam Related Compound B RS

2-Amino-2'-chloro-5-nitrobenzophenone.

$C_{13}H_9ClN_2O_3$  276.68

USP Clonazepam Related Compound C RS

2-Bromo-2'-(2-chlorobenzoyl)-4'-nitroacetanilide.

#### Identification, Infrared Absorption (197K).

**Melting range** (741): between 237° and 240°.

**Loss on drying** (731)—Dry it at 105° for 4 hours; it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

#### Delete the following:

• **Heavy metals, Method II** (231): 0.002%. • (Official 1-Jan-2018)

#### Limit of clonazepam related compound C—

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture.

*Test solution*—Dissolve an accurately weighed quantity of Clonazepam in acetone to obtain a solution having a concentration of 25 mg per mL.

*Standard solution*—Dissolve an accurately weighed quantity of USP Clonazepam Related Compound C RS in acetone to obtain a solution having a known concentration of 50 µg per mL.

Application volume: 20 µL.

Developing solvent system: a mixture of acetone and *n*-heptane (3:2).

*Procedure*—Proceed as directed for *Thin-Layer Chromatography* under *Chromatography* (621). After air-drying the plate, heavily spray the plate with 2 M sulfuric acid, and dry at 105° for 15 minutes. Successively spray the plate with 0.01 M sodium nitrite, 9 mM ammonium sulfamate, and *N*-(1-naphthyl)ethylenediamine dihydrochloride TS, and dry the plate with a current of air. Compare the intensities of any secondary spots observed in the chromatogram of the *Test solution* with that of the principal spot in the chromatogram of the *Standard solution*: no secondary spot from the chromatogram of the *Test solution* is larger or more intense than the principal spot obtained from the *Standard solution* (0.2%).

#### Related compounds—

*Buffer solution, Mobile phase, Diluent, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the Assay.

*Test preparation*—Use the Assay preparation.

*Procedure*—Inject a volume (about 50 µL) of the *Test preparation* into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of each impurity in the portion of Clonazepam taken by the formula:

$$100P_i / (r_c + \sum P_i)$$

in which *P* is the relative response factor, which is 1.84 for clonazepam related compound A, 0.94 for clonazepam related compound B, and 1 for all other impurities; *r<sub>i</sub>* is the peak response for each impurity obtained from the *Test preparation*; and *r<sub>c</sub>* is the peak response for clonazepam in the *Test preparation*: not more than 0.1% of clonazepam related compound A or of clonazepam related compound B is found, not more than 0.2% of any other impurity is found, and the sum of all other impurities is not more than 0.3%.



**Assay—**

**Buffer solution**—Transfer about 6.6 g of anhydrous dibasic ammonium phosphate to a 1-L volumetric flask, dissolve in 950 mL of water, adjust with 1 N phosphoric acid or 1 N sodium hydroxide to a pH of 8.0, dilute with water to volume, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution*, methanol, and tetrahydrofuran (60:52:13). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of water, methanol, and tetrahydrofuran (60:52:13).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Clonazepam RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.1 mg per mL.

**System suitability solution**—Dissolve suitable quantities of USP Clonazepam Related Compound A RS, USP Clonazepam Related Compound B RS, and USP Clonazepam RS in *Diluent* to obtain a solution containing about 0.04 mg per mL of each Reference Standard.

**Assay preparation**—Transfer about 10 mg of Clonazepam, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 2.2 for clonazepam related compound A, 2.5 for clonazepam related compound B, and 1.0 for clonazepam; and the resolution, *R*, between clonazepam related compound A and clonazepam related compound B is not less than 2.0. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{15}H_{10}ClN_3O_3$  in the portion of Clonazepam taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Clonazepam RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clonazepam Compounded Oral Suspension

**DEFINITION**

Clonazepam Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of clonazepam ( $C_{15}H_{10}ClN_3O_3$ ).

Prepare Clonazepam Compounded Oral Suspension 0.1 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Clonazepam	10 mg
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Vehicle: a 1:1 mixture of Vehicle for Oral Solution (regular or sugar-free), NF, and Vehicle for Oral Suspension, NF, a sufficient quantity to make	100 mL
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If using tablets, comminute the tablets into a fine powder in a suitable mortar, or add *Clonazepam* powder to the mortar. Add approximately 10 mL of the *Vehicle*, and mix to a uniform paste. Add the *Vehicle* in small portions almost to volume, and mix thoroughly after each addition. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough *Vehicle* to bring to final volume, and mix well.

**ASSAY****• PROCEDURE**

**Mobile phase:** Methanol, acetonitrile, and water (30:30:40). Filter and degas.

**Standard solution:** 25 µg/mL of USP Clonazepam RS in acetonitrile

**Sample solution:** Agitate the container of Oral Suspension for 30 min on a rotating mixer, remove a 5-mL sample, and store in a clear glass vial at -70° until analyzed. At the time of analysis, remove the sample from the freezer, allow it to reach room temperature, and mix with a vortex mixer for 30 s. Pipet 2.5 mL of the sample into a 10-mL volumetric flask, and dilute with acetonitrile to volume to obtain a solution having a nominal concentration of 25 µg/mL of clonazepam.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 10-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The retention time for clonazepam is about 7 min.]

**Suitability requirements**

**Relative standard deviation:** NMT 1.8% for replicate injections

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clonazepam ( $C_{15}H_{10}ClN_3O_3$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_U / r_S) \times (C_S / C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Clonazepam RS in the *Standard solution* (µg/mL)

*C<sub>U</sub>* = nominal concentration of clonazepam in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

**• PH (791):** 3.6–4.6

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature or in a refrigerator.

**• BEYOND-USE DATE:** NMT 60 days after the date on which it was compounded when stored at controlled room temperature or in a refrigerator

**• LABELING:** Label it to state that it is to be well shaken before use, and to state the *Beyond-Use Date*.



• **USP REFERENCE STANDARDS (11)**  
USP Clonazepam RS

## Clonazepam Tablets

» Clonazepam Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of clonazepam ( $C_{15}H_{10}ClN_3O_3$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, at room temperature.

**USP Reference standards (11)**—

USP Clonazepam RS

USP Clonazepam Related Compound A RS

3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyryl.

$C_{15}H_{10}ClN_3O_3$  315.72

USP Clonazepam Related Compound B RS

2-Amino-2'-chloro-5-nitrobenzophenone.

$C_{13}H_9ClN_2O_3$  276.68

**Identification**—

**A:** Place an amount of finely powdered Tablets, equivalent to about 10 mg of clonazepam, in a 125-mL separator. Add 25 mL of water, shake for 2 minutes, and extract with two 40-mL portions of chloroform. Pass the extracts through anhydrous sodium sulfate, combine them, and evaporate at room temperature with the aid of a stream of nitrogen to dryness. Wash the residue with three 10-mL portions of solvent hexane: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Clonazepam RS.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution (711)**—

*Medium:* degassed water; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 45 minutes.

Determine the amount of Clonazepam dissolved, using the following method.

**Mobile phase**—Prepare a filtered and degassed mixture of water, methanol, and acetonitrile (40:30:30). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Prepare a solution of USP Clonazepam RS in methanol having a known concentration of about 0.05 mg per mL. Quantitatively dilute a portion of this solution with *Dissolution Medium* to obtain a *Standard solution* having a known concentration similar to the expected concentration in the solution under test.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4-mm × 30-cm column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph replicate injections of the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%; and the tailing factor is not more than 2.0.

**Procedure**—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of  $C_{15}H_{10}ClN_3O_3$  dissolved by comparison of the peak responses obtained from the *Standard solution* and the test solution.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{15}H_{10}ClN_3O_3$  is dissolved in 45 minutes.

**Uniformity of dosage units (905):** meet the requirements.

**Related compounds**—

*Buffer solution, Mobile phase, Diluent, System suitability solution, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Clonazepam*.

*Test preparation*—Use the *Assay preparation*.

**Procedure**—Inject a volume (about 50 µL) of the *Test preparation* into the chromatograph, record the chromatogram, and measure the responses for all of the peaks. Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$100P_i / (r_c + \sum P_i)$$

in which *P* is the relative response factor, which is 2.45 for the peak with a relative retention time of 0.7 (if present), 1.84 for clonazepam related compound A, 0.94 for clonazepam related compound B, and 1 for all other impurities; *r<sub>i</sub>* is the peak response for each impurity obtained from the *Test preparation*; and *r<sub>c</sub>* is the peak response for clonazepam in the *Test preparation*: not more than 0.8% for the peak at relative retention time 0.7, not more than 0.4% of clonazepam related compound A, and not more than 1.0% of clonazepam related compound B are found; not more than 0.2% of any other impurity is found; and the sum of all other impurities is not more than 0.5%.

**Assay**—

*Buffer solution, Mobile phase, Diluent, Standard preparation, System suitability solution, and Chromatographic system*—Proceed as directed in the *Assay* under *Clonazepam*.

**Assay preparation**—Weigh and finely powder not fewer than 10 Tablets. Transfer an accurately weighed amount of powder, equivalent to about 10 mg of clonazepam, to a 100-mL volumetric flask; dissolve, with sonication, in 75 mL of *Diluent*; cool to room temperature, dilute with *Diluent* to volume, mix, and filter, discarding the first few mL of the filtrate.

**Procedure**—Separately inject equal volumes (about 50 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of clonazepam ( $C_{15}H_{10}ClN_3O_3$ ) in the portion of Tablets taken by the formula:

$$100C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Clonazepam RS in the *Standard preparation*; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clonazepam Orally Disintegrating Tablets

» Clonazepam Orally Disintegrating Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of clonazepam  $C_{15}H_{10}ClN_3O_3$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers, and store at controlled room temperature.

**USP Reference standards (11)**—

USP Clonazepam RS

USP Clonazepam Related Compound A RS



3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyryl.

$C_{15}H_{10}ClN_3O_3$  315.72

USP Clonazepam Related Compound B RS

2-Amino-2'-chloro-5-nitrobenzophenone.

$C_{13}H_9ClN_2O_3$  276.68

#### Identification—

**A:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Disintegration** (701): not more than 60 seconds.

#### Dissolution (711)—

*Medium:* water; 900 mL, degassed.

*Apparatus 2:* 50 rpm.

*Time:* 60 minutes.

*Mobile phase, Standard preparation, and Chromatographic system*—Prepare as directed in the *Assay*.

*Standard solution*—Quantitatively dilute with *Medium* the *Standard preparation* as directed in the *Assay* according to the Tablet strength. The final concentration of the *Standard solution* corresponding to each Tablet strength is given in Table 1.

Table 1

Tablet Strength mg per Tablet	Final Standard solution µg/mL of Clonazepam
0.125	0.125
0.25	0.25
0.5	0.50
1.0	1.0
2.0	2.0

*Test solution*—Pass a portion of the solution under test through a 0.45-µm nylon membrane filter, discarding the first few mL.

*Procedure*—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the clonazepam peak. Calculate the percentage of clonazepam dissolved by the formula:

$$\frac{r_U \times C_S \times 900 \times 100}{r_S \times L}$$

in which  $r_U$  and  $r_S$  are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration of USP Clonazepam RS, in mg per mL, in the *Standard solution*; 900 is the volume of the *Medium*; and  $L$  is the Tablet label claim, in mg.

**Tolerances**—Not less than 75% (Q) of the labeled amount of clonazepam is dissolved in 60 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Related compounds—

*Mobile phase and Standard preparation*—Prepare as directed in the *Assay*.

*System suitability solution*—Dissolve weighed quantities of USP Clonazepam Related Compound A RS and USP Clonazepam Related Compound B RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 µg per mL each of USP Clonazepam Related Compound A RS and USP Clonazepam Related Compound B RS.

*Standard solution*—Quantitatively dilute with *Mobile phase* the *Standard preparation* to obtain a solution having a known concentration of about 0.2 µg per mL of clonazepam.

*Test solution*—Weigh and finely powder not fewer than 20 Tablets. Grind the Tablets into a fine powder and transfer

an accurately weighed portion of the powder, equivalent to about 2 mg of clonazepam, to a 50-mL volumetric flask, pipet 20.0 mL of *Mobile phase* into the flask, and sonicate for about 2 minutes with intermittent shaking. DO NOT dilute to volume. Shake the flask for 30 minutes on a mechanical shaker. Pass a portion of this solution through a nylon membrane filter having a 0.45-µm or finer porosity, and use the filtrate after discarding the first 4 mL of the filtrate.

*Chromatographic system* (see *Chromatography* (621))—Prepare as directed in the *Assay*. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*. Identify the peaks due to clonazepam related compound A and clonazepam related compound B using the relative retention times given in Table 2; the resolution,  $R$ , between clonazepam related compound A and clonazepam related compound B is not less than 2.0. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.0, and the relative standard deviation for six replicate injections is not more than 6.0%.

*Procedure*—Separately inject equal volumes (about 100 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for at least four times the retention time of clonazepam, and measure the responses for all the peaks. [NOTE—Disregard any peaks with a retention time less than 3.5 minutes.] Calculate the percentage of each impurity in the portion of Tablets taken by the formula:

$$(C_S / C_T)(r_T / r_S)(1 / F)100$$

in which  $C_S$  is the concentration, in µg per mL, of USP Clonazepam RS in the *Standard solution*;  $C_T$  is the nominal concentration, in µg per mL, of clonazepam in the *Test solution*;  $r_T$  is the peak response of each impurity obtained from the *Test solution*;  $r_S$  is the peak response for clonazepam obtained from the *Standard solution*; and  $F$  is the relative response factor for the impurity given in Table 2. The limits of each impurity along with relative retention times and relative response factors are given in Table 2.

Table 2

Peak ID	Relative Retention Time	Relative Response Factor	Limit % (w/w)
Clonazepam	1.0	1.0	—
Clonazepam related compound A <sup>1</sup>	1.71	0.67	0.4
Clonazepam related compound B <sup>2</sup>	2.25	0.79	1.0
Any other unspecified degradation product	—	1.0	0.2
Total impurities	—	—	2.0

<sup>1</sup>3-Amino-4-(2-chlorophenyl)-6-nitrocarbostyryl.

<sup>2</sup>2-Amino-2'-chloro-5-nitrobenzophenone.

#### Assay—

*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and methanol (2:1:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard preparation*—Dissolve an accurately weighed quantity of USP Clonazepam RS in *Mobile phase*, and dilute, if necessary, to obtain a solution having a known concentration of about 0.01 mg per mL of USP Clonazepam RS.

*Assay preparation*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 2 mg of clonazepam, to a 200-mL volumetric flask, add 120 mL of *Mobile phase*, and sonicate for about 15 minutes with intermittent shaking. Shake the flask on a mechanical shaker for about 30 min-



utes. Dilute with *Mobile phase* to volume, and mix. Pass a portion of this solution through a nylon membrane filter having a 0.45- $\mu$ m or finer porosity, and use the filtrate after discarding the first 4 mL of the filtrate. [NOTE—The solution is stable for 48 hours at room temperature.]

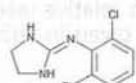
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L7. The flow rate is about 1.2 mL per minute. The column temperature is maintained at 30°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*; the column efficiency is not fewer than 2000 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 60  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the clonazepam peak. Calculate the quantity, in percentage, of label claim of clonazepam ( $C_{15}H_{10}ClN_3O_3$ ) in the portion of Tablets taken by the formula:

$$(C_s / C_u)(r_u / r_s)100$$

in which  $C_s$  is the concentration, in mg per mL, of USP Clonazepam RS in the *Standard solution*;  $C_u$  is the nominal concentration based on label claim, in mg per mL, of clonazepam in the *Assay preparation*;  $r_u$  is the peak response for clonazepam obtained from the *Assay preparation*; and  $r_s$  is the peak response for clonazepam obtained from the *Standard preparation*.

## Clonidine



$C_9H_9Cl_2N_3$  230.09  
Benzenamine, 2,6-dichloro-N-2-imidazolidinylidene-;  
2-[(2,6-Dichlorophenyl)imino]imidazolidine [4205-90-7].

### DEFINITION

Clonidine contains NLT 99.0% and NMT 101.0% of clonidine ( $C_9H_9Cl_2N_3$ ), calculated on the dried basis.

### IDENTIFICATION

- A. INFRARED ABSORPTION** (197K)
- B. ULTRAVIOLET ABSORPTION** (197U)

**Medium:** 0.01 N hydrochloric acid

**Sample solution:** 0.3 mg/mL of Clonidine in *Medium*

**Acceptance criteria:** Absorptivities are about 2.1 and 1.8 for the maxima at 271 nm and 279 nm, respectively, calculated on the dried basis.

### ASSAY

#### PROCEDURE

**Sample solution:** 190 mg of Clonidine in 100 mL of glacial acetic acid

**Analysis:** Titrate with 0.1 N perchloric acid VS, using a silver-silver chloride glass combination electrode with liquid junction. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each mL of 0.1 N perchloric acid is equivalent to 23.01 mg of clonidine ( $C_9H_9Cl_2N_3$ ).

**Acceptance criteria:** 99.0%–101.0% on the dried basis

### IMPURITIES

#### RESIDUE ON IGNITION (281)

**Analysis:** Ignite at  $500 \pm 25^\circ$  to constant weight.

**Acceptance criteria:** NMT 0.1%

#### Delete the following:

- HEAVY METALS, Method II (231):** NMT 10  $\mu$ g/g (Official 1-

Jan-2018)

#### ORGANIC IMPURITIES

**1 M phosphoric acid:** 115 g/L of phosphoric acid in water

**Buffer:** 4 g/L of monobasic potassium phosphate in water. Adjust with 1 M phosphoric acid to a pH of 4.0.

**Solution A:** Acetonitrile and Buffer (75:25)

**Mobile phase:** See Table 1.

Table 1

Time (min)	Buffer (%)	Solution A (%)
0	90	10
15	30	70
15.1	90	10
20	90	10

**Acetylclonidine solution:** 0.05 mg/mL of USP Clonidine Related Compound A RS, prepared as follows. Initially dissolve USP Clonidine Related Compound A RS in acetonitrile (1 mg/mL), and dilute with Buffer to volume.

**System suitability solution:** 0.86 mg/mL of USP Clonidine RS and 0.86  $\mu$ g/mL of clonidine related compound A from *Acetylclonidine solution* in Buffer

**Standard solution:** 8.6  $\mu$ g/mL of USP Clonidine RS in Buffer

**Sample solution:** 0.86 mg/mL of Clonidine in Buffer

**Blank:** Buffer

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 3.0-mm  $\times$  15-cm; 5- $\mu$ m packing L56

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 5  $\mu$ L

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 5 between clonidine and acetylclonidine, *System suitability solution*

**Tailing factor:** NMT 2.5 for clonidine, *System suitability solution*

**Relative standard deviation:** NMT 5%, *Standard solution*

#### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
Calculate the percentage of each impurity in the portion of Clonidine taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times 100$$

$r_u$  = peak response for each impurity from the *Sample solution*

$r_s$  = peak response of clonidine from the *Standard solution*

$C_s$  = concentration of the *Standard solution* ( $\mu$ g/mL)

$C_u$  = concentration of the *Sample solution* ( $\mu$ g/mL)



**Acceptance criteria**

Individual impurity: NMT 0.1%

Total impurities: NMT 0.2%

**SPECIFIC TESTS**• **LOSS ON DRYING (731)**

Analysis: Dry a sample at 60° under vacuum to constant weight.

Acceptance criteria: NMT 0.5%

• **APPEARANCE OF SOLUTION****Color of solution**

Standard solution: 4.8 µg/mL of potassium chromate in water

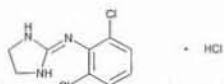
Sample solution: 100 mg/mL in methanol

Acceptance criteria: The color of 10 mL of *Sample solution* is not more intense than that of 10 mL of *Standard solution* when compared in matched color-comparison tubes (see *Nephelometry, Turbidimetry, and Visual Comparison (855), Visual Comparison*).**Turbidity**Sample solution: Prepare as directed in *Color of solution*.Acceptance criteria: 10 mL of *Sample solution* has no more turbidity than 10 mL of methanol when compared in matched color-comparison tubes (see *Nephelometry, Turbidimetry, and Visual Comparison (855), Visual Comparison*).**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature.• **USP REFERENCE STANDARDS (11)**

USP Clonidine RS

USP Clonidine Related Compound A RS

1-Acetyl-2-(2,6-dichlorophenylamino)-2-(4,5-dihydroimidazol).

C<sub>11</sub>H<sub>11</sub>Cl<sub>2</sub>N<sub>3</sub>O 272.13**Clonidine Hydrochloride**C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub> · HCl 266.55

Benzenamine, 2,6-dichloro-N-2-imidazolidinylidene-, monohydrochloride;

2-[(2,6-Dichlorophenyl)imino]imidazolidine monohydrochloride [4205-91-8].

**DEFINITION**Clonidine Hydrochloride contains NLT 98.5% and NMT 101.0% of clonidine hydrochloride (C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub> · HCl), calculated on the dried basis.**IDENTIFICATION**• **A. INFRARED ABSORPTION (197K)**• **B. ULTRAVIOLET ABSORPTION (197U)**

Analytical wavelength: 272 nm

Medium: 0.01 N hydrochloric acid

Sample solution: 330 µg/mL

Acceptance criteria: Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

• **C. IDENTIFICATION TESTS—GENERAL, Chloride (191):** Meets the requirements**ASSAY**• **PROCEDURE**

Solution A: 1.0 mL/L of triethylamine in water

Mobile phase: Acetonitrile and *Solution A* (32:68). Adjust with phosphoric acid to a pH of 6.9.System suitability solution: 0.05 mg/mL each of USP Clonidine Hydrochloride RS and USP Clonidine Related Compound A RS in *Mobile Phase*. Pass through a suitable filter of 0.45-µm pore size.Standard solution: 0.05 mg/mL of USP Clonidine Hydrochloride RS in *Mobile phase*. Pass through a suitable filter of 0.45-µm pore size.Sample solution: 0.05 mg/mL of Clonidine Hydrochloride in *Mobile phase*. Pass through a suitable filter of 0.45-µm pore size.**Chromatographic system**(See *Chromatography (621), System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 2 mL/min

Injection size: 50 µL

**System suitability**Samples: *System suitability solution* and *Standard solution***Suitability requirements**Resolution: NLT 2.0 between clonidine and clonidine related compound A, *System suitability solution*Tailing factor: NMT 2.0, *Standard solution*Relative standard deviation: NMT 1.0%, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of clonidine hydrochloride (C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub> · HCl) in the portion of Clonidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Clonidine Hydrochloride RS in the *Standard solution* (mg/mL) $C_U$  = concentration of Clonidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.0% on the dried basis

**IMPURITIES**• **RESIDUE ON IGNITION (281):** NMT 0.1%• **ORGANIC IMPURITIES**

Solution A and Mobile phase: Proceed as directed in the Assay.

Standard stock solution: 0.06 mg/mL each of USP Clonidine Hydrochloride RS, USP Clonidine Related Compound A RS, and 2,6-dichloroaniline in acetonitrile. [NOTE—The *Standard stock solution* is stable for 30 days when protected from light and stored in a refrigerator.]Standard solution: 0.6 µg/mL of USP Clonidine Hydrochloride RS in *Mobile phase* from the *Standard stock solution*. Pass through a suitable filter of 0.45-µm pore size.Sample solution: 0.15 mg/mL of Clonidine Hydrochloride in *Mobile phase*. Initially add *Mobile phase* to about 60% of the volume of the flask, sonicate for 5 min, and then dilute with *Mobile phase* to volume. Pass through a suitable filter of 0.45-µm pore size.**Chromatographic system**(See *Chromatography (621), System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 3.9-mm × 30-cm; 10-µm packing L1

Flow rate: 2 mL/min

Injection size: 50 µL

Run time: 6.6 times the retention time of the clonidine peak

**System suitability**Sample: *Standard solution***Suitability requirements**

Resolution: NLT 2.0 between clonidine related compound A and 2,6-dichloroaniline



Capacity factor,  $k'$ : NLT 1.7 for all peaks

Relative standard deviation: NMT 5% for all peaks

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of clonidine related compound A and 2,6-dichloroaniline in the portion of Clonidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of clonidine related compound A or 2,6-dichloroaniline from the *Sample solution*  
 $r_S$  = peak response of clonidine related compound A or 2,6-dichloroaniline from the *Standard solution*  
 $C_S$  = concentration of USP Clonidine Related Compound A RS or 2,6-dichloroaniline in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Clonidine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other unspecified impurity in the portion of Clonidine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of any other unspecified impurity from the *Sample solution*  
 $r_S$  = peak response of clonidine from the *Standard solution*  
 $C_S$  = concentration of USP Clonidine Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Clonidine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1. Disregard any impurity less than or equal to 0.04%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Clonidine hydrochloride	1.0	—
Clonidine related compound A <sup>a</sup>	2.5	0.1
2,6-Dichloroaniline	3.3	0.1
Any other unspecified impurity	—	0.1
Total impurities <sup>b</sup>	—	0.2

<sup>a</sup> 1-Acetyl-2-(2,6-dichlorophenylimino)-imidazolidine.

#### SPECIFIC TESTS

##### • PH (791)

Sample solution: 50 mg/mL

Acceptance criteria: 3.5–5.5

##### • LOSS ON DRYING (731)

Analysis: Dry a sample at 105° to constant weight.

Acceptance criteria: NMT 0.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

##### • USP REFERENCE STANDARDS (11)

USP Clonidine Hydrochloride RS

USP Clonidine Related Compound A RS

1-Acetyl-2-(2,6-dichlorophenylimino)-imidazolidine.

C<sub>11</sub>H<sub>11</sub>Cl<sub>2</sub>N<sub>3</sub>O 272.13

## Clonidine Hydrochloride Tablets

### DEFINITION

Clonidine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clonidine hydrochloride (C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub> · HCl).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

##### • B. THIN-LAYER CHROMATOGRAPHY

Standard solution: 10 mg/mL of USP Clonidine Hydrochloride RS in methanol

Sample solution: Transfer an equivalent to 1 mg of clonidine hydrochloride, from a quantity of finely powdered Tablets, to a separator containing 30 mL of water and 5 mL of 1 N sodium hydroxide. Swirl gently to dissolve the sample specimen, and extract with 20 mL of chloroform. Allow the layers to separate, and filter the chloroform extract. Evaporate the filtrate to dryness, and dissolve the residue in 0.1 mL of methanol.

#### Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 2  $\mu$ L

Developing solvent system: Methanol and ammonium hydroxide (200:3)

#### Analysis

Samples: *Standard solution* and *Sample solution*

Proceed as directed in the chapter. Position the plate in a chromatographic chamber, and develop in *Developing solvent system* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Examine the plate under short-wavelength UV light.

Acceptance criteria: The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

### ASSAY

#### • PROCEDURE

Solution A: 2.2 mg/mL of sodium 1-octanesulfonate in water

Mobile phase: Methanol, *Solution A*, and phosphoric acid (500:500:1). Adjust with 1 N sodium hydroxide to a pH of 3.0, and pass through a suitable filter of 0.45- $\mu$ m pore size.

2,6-Dichloroaniline stock solution: 12  $\mu$ g/mL of 2,6-dichloroaniline in *Mobile phase*

Standard stock solution: 100  $\mu$ g/mL of USP Clonidine Hydrochloride RS in *Mobile phase*

Standard solution: 1  $\mu$ g/mL of clonidine hydrochloride from the *Standard stock solution* in *Mobile phase*

System suitability solution: 2  $\mu$ g/mL of USP Clonidine Hydrochloride RS and 2.4  $\mu$ g/mL of 2,6-dichloroaniline in *Mobile phase*, from the *Standard stock solution* and 2,6-Dichloroaniline stock solution, respectively

Sample solution: Weigh and finely powder NLT 20 Tablets. Transfer the equivalent to 0.1 mg of clonidine hydrochloride from powder to a 100-mL volumetric flask. Add about 60 mL of *Mobile phase*, shake by mechanical means for 15–30 min, dilute with *Mobile phase* to volume, and mix. Centrifuge a portion of this solution to obtain a clear solution.

#### Chromatographic system

(See Chromatography (621), System Suitability.)



Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; packing L7, deactivated for basic compounds

Flow rate: 1.5 mL/min

Injection volume: 50 µL

#### System suitability

Samples: *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clonidine and 2,6-dichloroaniline are about 0.5 and 1.0, respectively.]

#### Suitability requirements

Tailing factor: NMT 1.5 for the clonidine peak, *System suitability solution*

Column efficiency: NLT 3500 theoretical plates for the clonidine peak, *System suitability solution*

Relative standard deviation: NMT 2.0% for the clonidine peak, *Standard solution*

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clonidine hydrochloride ( $C_9H_9Cl_2N_3 \cdot HCl$ ) in the portion of sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of clonidine hydrochloride in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

Medium: 0.01 N hydrochloric acid; 500 mL

Apparatus 2: 50 rpm

Time: 30 min

Standard solution: Proceed as directed in the Assay, except use *Medium* instead of *Mobile phase*.

Analysis: Proceed as directed in the Assay.

Tolerances: NLT 75% (Q) of the labeled amount of clonidine hydrochloride ( $C_9H_9Cl_2N_3 \cdot HCl$ ) is dissolved.

##### • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE: Preserve in well-closed containers.

##### • USP REFERENCE STANDARDS (11)

USP Clonidine Hydrochloride RS

## Clonidine Hydrochloride and Chlorthalidone Tablets

#### DEFINITION

Clonidine Hydrochloride and Chlorthalidone Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) and NLT 90.0% and NMT 110.0% of the labeled amount of clonidine hydrochloride ( $C_9H_9Cl_2N_3 \cdot HCl$ ).

#### IDENTIFICATION

##### • A.

**Sample solution:** Transfer an amount of powdered Tablets, equivalent to 3 mg of clonidine hydrochloride, to a beaker. Add 30 mL of water, stir for 5 min, and pass through a filter of medium pore size into a sintered-glass funnel. Transfer the filtrate to a separator, add 5 mL of 0.1 N sodium hydroxide, and extract with 20 mL of chloroform, collecting the chloroform extract

in a separator. Extract the chloroform phase with 15 mL of 0.01 N hydrochloric acid, collecting the acid extract in a beaker. Remove any residual chloroform from the acid extract by heating on a steam bath.

**Acceptance criteria:** The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Clonidine Hydrochloride RS, concomitantly measured.

##### • B. INFRARED ABSORPTION

**Sample:** Transfer 10 powdered Tablets to a 50-mL beaker. Add 10 mL of methanol, boil on a steam bath for 5 min, and filter. Add 20 mL of water to the filtrate, and boil on a steam bath for 5 min under a current of air. Cool, with stirring, in ice until crystals form. Filter the crystals, and dry at 105° for 1 h.

**Acceptance criteria:** The IR absorption spectrum of a mineral oil dispersion of the *Sample* exhibits maxima only at the same wavelengths as that of a similar preparation of USP Chlorthalidone RS.

##### • C. The retention times of the chlorthalidone and clonidine hydrochloride peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Buffer:** 1 g/L of monobasic ammonium phosphate in water

**Mobile phase:** Methanol, acetonitrile, and *Buffer* (100:100:800)

**Clonidine hydrochloride standard stock solution:**

1500 µg/mL of USP Clonidine Hydrochloride RS in *Buffer*;  $J$  is the ratio of the labeled amount, in mg, of clonidine hydrochloride to the labeled amount, in mg, of chlorthalidone per Tablet.

**Standard solution:** 150 µg/mL of USP Clonidine Hydrochloride RS and 150 µg/mL of USP Chlorthalidone RS prepared as follows. Transfer 15 mg of USP Chlorthalidone RS to a 100-mL volumetric flask, dissolve in 10 mL of methanol, and add 25 mL of *Buffer* and 10.0 mL of *Clonidine hydrochloride standard stock solution*. Dilute with *Buffer* to volume.

**Sample solution:** Transfer an amount equivalent to 15 mg of chlorthalidone from powdered Tablets (NLT 20). Add 10 mL of methanol, and sonicate for 5 min. Add 40 mL of *Buffer*, and sonicate until the solution is free from agglomerates. Allow to cool to ambient temperature, dilute with *Buffer* to volume, and centrifuge.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 10-cm; packing L7

Flow rate: 2 mL/min

Injection volume: 20 µL

#### System suitability

Samples: *Standard solution*

[NOTE—The relative retention times for clonidine hydrochloride and chlorthalidone are about 0.2 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 3 between the clonidine hydrochloride and chlorthalidone peaks

**Relative standard deviation:** NMT 2%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amounts of clonidine hydrochloride ( $C_9H_9Cl_2N_3 \cdot HCl$ ) and chlorthalidone ( $C_{14}H_{11}ClN_2O_4S$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clonidine hydrochloride or chlorthalidone from the *Sample solution*



- $r_s$  = peak response of clonidine hydrochloride or chlorthalidone from the *Standard solution*  
 $C_s$  = concentration of USP Clonidine Hydrochloride RS or USP Chlorthalidone RS in the *Standard solution* ( $\mu\text{g/mL}$ )  
 $C_u$  = nominal concentration of clonidine hydrochloride or chlorthalidone in the *Sample solution* ( $\mu\text{g/mL}$ )  
 Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### • DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 100 rpm

Time: 60 min

**Sample solution:** Pipet 20 mL of a centrifuged portion of the solution under test into a 25-mL volumetric flask, and dilute with 0.5% monobasic ammonium phosphate solution to volume. Use the resulting solution as the *Sample solution*.

**Analysis:** Proceed as directed in the *Assay*, making any necessary volumetric adjustments.

**Tolerances:** NLT 50% (Q) of the labeled amount of chlorthalidone ( $\text{C}_{14}\text{H}_{11}\text{ClN}_2\text{O}_4\text{S}$ ) and NLT 80% (Q) of the labeled amount of clonidine hydrochloride ( $\text{C}_9\text{H}_9\text{Cl}_2\text{N}_3 \cdot \text{HCl}$ ) are dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Content Uniformity* with respect to both clonidine hydrochloride and chlorthalidone

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

### • USP REFERENCE STANDARDS (11)

USP Chlorthalidone RS

USP Clonidine Hydrochloride RS

## Clonidine Transdermal System

### DEFINITION

Clonidine Transdermal System contains NLT 80.0% and NMT 120.0% of the labeled amount of clonidine ( $\text{C}_9\text{H}_9\text{Cl}_2\text{N}_3$ ).

[NOTE—Throughout the following procedures, avoid the use of tetrahydrofuran stabilized with butylated hydroxytoluene (BHT). In the presence of peroxides, BHT may react with clonidine, producing impurity peaks.]

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

**Buffer solution:** 242.28 g/L of tris-(hydroxymethyl)aminomethane in water. Adjust with dilute hydrochloric acid to a pH of 9.2.

**Sample:** Carefully peel the release liner from each Transdermal System, and place a number of Transdermal Systems equivalent to 25 mg of clonidine into a 50-mL screw-capped centrifuge tube. Add 5 mL of chloroform, and mix on a vortex mixer for 5 min. Allow to stand for 30 min, and mix intermittently on a vortex mixer. Transfer the chloroform solution to another 50-mL centrifuge tube, and wash the residue with an additional 3 mL of chloroform, combining the extracts. Add 2 mL of 0.5 N hydrochloric acid to the extract, mix on a vortex mixer for 1 min, and centrifuge at about 1000 rpm for 4 min. Remove and discard the bottom chloroform layer. Extract the aqueous layer with 4 mL of chloroform. Centrifuge at 1000 rpm for an additional 5 min, and again discard the bottom chloroform layer. Add 5 mL of *Buffer solution* and 3 mL of methylene chloride. Mix on a vortex mixer for 1 min. Centrifuge at 1000 rpm for 4 min. Transfer the bottom methylene chloride layer into a 100-mL beaker, and dry the meth-

ylene chloride with anhydrous sodium sulfate (about 1/4 liquid height). Decant, and evaporate to dryness with a stream of nitrogen. Dry at 105° for 30 min, and allow to cool in a desiccator.

**Analysis:** Determine the IR spectrum of the *Sample solution* and USP Clonidine RS in the wavelength region of 3500–600  $\text{cm}^{-1}$ .

**Acceptance criteria:** Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

## ASSAY

### • PROCEDURE

**Buffer solution:** 2.5 mL of triethylamine in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Acetonitrile and *Buffer solution* (60:40).

[NOTE—Stir the solution for 30 min.]

**Diluent:** Tetrahydrofuran and methanol (1:1)

**System suitability solution:** 250  $\mu\text{g/mL}$  of USP Clonidine RS and 10  $\mu\text{g/mL}$  of USP Clonidine Related Compound B RS in *Diluent*

**Standard stock solution:** 1 mg/mL of USP Clonidine RS in tetrahydrofuran

**Standard solutions:** Prepare a minimum of four *Standard solutions* from the *Standard stock solution* in *Diluent* that bracket the expected clonidine concentration in the sample. The standard concentrations should be within the range of 50–300  $\mu\text{g/mL}$ . [NOTE—The *Standard solutions* are stable for up to 2 days if stored at 4°.]

**Sample solution:** 357  $\mu\text{g/mL}$  of clonidine prepared as follows. Remove each Transdermal System from its package, discard the release liner from each system, and transfer into a 50-mL centrifuge tube with a Teflon-lined screw cap. Add the appropriate volume of tetrahydrofuran as listed in *Table 1*.

Table 1

For systems containing about 2.5 mg of clonidine	7.0 mL
For systems containing about 5.0 mg of clonidine	14.0 mL
For systems containing about 7.5 mg of clonidine	21.0 mL

Mix vigorously on a vortex mixer until the systems are washed down and fully submerged in the tetrahydrofuran. Let the systems soak in tetrahydrofuran for about 5 min, and mix on a vortex mixer until the systems are completely delaminated. Allow the systems to remain submerged for an additional 60 min, mixing on a vortex mixer every 30 min. Add methanol in a volume equal to the volume of tetrahydrofuran, and mix vigorously on a vortex mixer. The solution turns milky. Centrifuge for 10 min at 2000 rpm. Use the supernatant as the *Sample solution*.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 and 242 nm

[NOTE—The detector is programmed initially to 242 nm and switched to 210 nm after the elution of the clonidine peak but before the elution of the clonidine related compound B peak.]

**Column:** 4.6-mm  $\times$  15-cm; packing L10

**Flow rate:** 1 mL/min

**Injection size:** 25  $\mu\text{L}$

### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for clonidine and clonidine related compound B are 1.0 and 1.5, respectively.]

### Suitability requirements

**Resolution:** NLT 2.0 between clonidine and clonidine related compound B



**Capacity factor (*k'*):** NLT 0.6 for clonidine  
**Tailing factor:** NMT 3.0 for both clonidine and clonidine related compound B  
**Relative standard deviation:** NMT 2.0% for the clonidine peak area

#### Analysis

**Samples:** At least three *Standard solutions* that will bracket the expected sample concentration range and the *Sample solution*

Calculate the peak response ratios of the analyte, and plot the results. Determine the linear regression equation of the standards by the mean-square method, and record the linear regression equation and the correlation coefficient: it should be NLT 0.995.

Calculate the percentage of the labeled amount of clonidine ( $C_9H_9Cl_2N_3$ ) in the Transdermal System taken:

$$\text{Result} = (C_s/C_u) \times 100$$

$C_s$  = concentration of clonidine from the linear regression analysis ( $\mu\text{g/mL}$ )

$C_u$  = nominal concentration of clonidine in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria:** 80.0%–120.0%

#### PERFORMANCE TESTS

##### • DRUG RELEASE (724)

###### Test 1

**Medium:** 0.001 M phosphoric acid; 80 mL for systems containing 5 mg or less of clonidine; 200 mL for systems containing more than 5 mg of clonidine

**Times:** 8, 24, 96, and 168 h

**Apparatus 7:** Proceed as directed in the chapter, using the transdermal system holder-angled disk (see *Figure 4a*). The appropriate size of the holder, 1.42 or 1.98 inches, should be chosen based on the size of the system to prevent overhang. Use 100-mL beakers for *Medium* volumes of 80 mL and 300-mL beakers for *Medium* volumes of 200 mL. Gently press the Transdermal System to a dry, smooth, square piece of cellulose membrane, or equivalent, with the adhesive side against the membrane. Attach the membrane/system to a suitable inert sample holder with a Viton O-ring, or equivalent, so that the backing of the system is adjacent to and centered on the bottom of the sample holder. Trim the excess cellulose membrane with scissors. Suspend each sample holder from the arm of a reciprocating shaker so that each system is continuously immersed in a beaker containing the specified volume of *Medium*. The filled beakers are weighed and pre-equilibrated to  $32.0 \pm 0.3^\circ$  before immersing the test sample. Agitate the sample in an up-down motion at a frequency of 30 cycles/min with an amplitude of  $2.0 \pm 0.1$  cm. The *Medium* must be added daily to the beakers during each interval to maintain sample immersion. At the end of each time interval, transfer the test sample to a fresh beaker containing the appropriate volume of *Medium*, weighed and pre-equilibrated to  $32.0 \pm 0.3^\circ$ .

**Mobile phase:** 0.1% solution of triethylamine in a mixture of methanol and water (30:70). Adjust with phosphoric acid to a pH of  $6.0 \pm 0.2$ .

**System suitability solution:** 10  $\mu\text{g/mL}$  of USP Clonidine RS in 0.001 M phosphoric acid

**Standard solutions:** Prepare a minimum of four *Standard solutions* of USP Clonidine RS in 0.001 M phosphoric acid having known concentrations of clonidine similar to those of the *Sample solutions*.

**Sample solutions:** At the end of each release interval, allow the beakers to cool to room temperature, and make up for evaporative *Medium* losses by adding *Medium* to obtain the original weight, then mix.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 25  $\mu\text{L}$

#### System suitability

**Sample:** *System suitability solution*

#### Suitability requirements

**Column efficiency:** NLT 2000 theoretical plates

**Tailing factor:** NMT 2.0

**Capacity factor (*k'*):** NLT 0.5

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solutions* and *Sample solutions*

Construct a standard curve of concentration ( $\mu\text{g/mL}$ ) of clonidine in the *Standard solutions* versus peak area by linear regression analysis. The correlation coefficient is NLT 0.995.

Calculate the release rate of clonidine:

$$\text{Result} = CV/TA$$

$C$  = concentration of clonidine in the sample of the standard curve ( $\mu\text{g/mL}$ )

$V$  = volume of the *Medium* (mL)

$T$  = time (h)

$A$  = area of the Transdermal System ( $\text{cm}^2$ )

**Tolerances:** See *Table 2*.

**Table 2**

Time (h)	Time for Sampling (h)	Release Rate ( $\mu\text{g/h/cm}^2$ )
0–8	8	7.5–16.0
8–24	24	1.5–4.6
24–96	96	1.5–4.6
96–168	168	1.5–3.3

The release rate of clonidine ( $C_9H_9Cl_2N_3$ ) from the Transdermal System, expressed as  $\mu\text{g/h/cm}^2$  at the times specified, conforms to *Acceptance Table 1* in (724).

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Drug Release Test 2*.

**Medium:** 0.01 N hydrochloric acid; 500 mL for systems labeled as 0.1 mg/day, 900 mL for systems labeled as 0.2 or 0.3 mg/day

**Apparatus 6:** 100 rpm. Apply double-sided tape around the lower-most circumference of the cylinder, overlapping the ends to prevent peeling of the tape end from the cylinder. Remove the outer layer of the tape. Attach the Transdermal System to the cylinder with the backing side against the double-sided tape and the longitudinal axis parallel to the bottom of the cylinder. Carefully smooth the system to remove any air bubbles, and remove the release liner from the system. For systems requiring 500 mL of *Medium*, apply the double-sided tape to the system such that the bottom edge of each is NMT 2 mm from the bottom of the cylinder to prevent evaporation during the test from exposure to air. After setting the cylinder in the vessel, cover the vessel to minimize evaporation.

**Times:** 6, 48, 96, and 168 h

**Buffer:** 0.3% triethylamine in 0.025 M monobasic potassium phosphate. Adjust with phosphoric acid to a pH of  $6.20 \pm 0.10$ .

**Mobile phase:** *Buffer* and tetrahydrofuran (94:6)

**Standard solutions:** Solutions containing 0.7, 3.0, 5.3, 7.5, and 9.8  $\mu\text{g/mL}$  of USP Clonidine RS in *Medium*. A small amount of methanol (not exceeding 10% of the final volume) can be used to solubilize clonidine.

**Sample solution:** 1.5 mL aliquots of the solution under test. After sampling the last time point, measure the volume of *Medium* remaining in the vessel.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 210 nm**Columns**

Guard: 3.0-mm × 4-mm; packing L1

Analytical: 4.6-mm × 15-cm; packing L1

**Flow rate:** 1.0 mL/min**Injection size:** 50 µL**System suitability****Sample:** 5.3 µg/mL of the *Standard solution***Suitability requirements****Tailing factor:** NMT 2.0**Relative standard deviation:** NMT 3.0%**Analysis****Samples:** *Standard solutions* and *Sample solutions*Construct a standard curve of concentration (µg/mL) of clonidine in the *Standard solutions* versus peak area by linear regression analysis. The correlation coefficient is NLT 0.997. Calculate the release rate of clonidine.Calculate the volume loss rate in mL/h (*L*):

$$L = [V - F + (N \times 1.5)]/T$$

*V* = initial volume of *Medium* (mL)*F* = final volume of *Medium* (mL)*N* = number of sampling time points*T* = total elapsed time between start of run and final volume measurement (h)Calculate the volume (mL) at each sampling time adjusted for evaporation (*V<sub>adj</sub>*):

$$V_{adj} = V - (L \times t_c) - [(n - 1) \times 1.5]$$

*t<sub>c</sub>* = cumulative time for the sample withdrawal (6, 48, 96, or 168 h)*n* = sampling number (1, 2, 3, or 4 for the 6-, 48-, 96-, and 168-h sampling times, respectively)Calculate the release rate of clonidine (µg/h/cm<sup>2</sup>):

$$\text{Result} = [(r_u - b) \times V_{adj}]/(m \times A \times t_i)$$

*r<sub>u</sub>* = peak response from the *Sample solution**b* = y-intercept of the standard curve*m* = slope of the standard curve*A* = area of the system (cm<sup>2</sup>)*t<sub>i</sub>* = interval time (h)**Tolerances:** See *Table 3*.**Table 3**

Time (h)	Time for Sampling (h)	Interval Time (h)	Release Rate (µg/h/cm <sup>2</sup> )
0-6	6	6	7.6-12.0
6-48	48	42	1.7-2.5
48-96	96	48	2.0-2.9
96-168	168	72	1.7-2.6

The release rate of clonidine (C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>) from the Transdermal System, expressed as µg/h/cm<sup>2</sup> at the times specified, conforms to *Acceptance Table 1* in (724).**Test 3:** If the product complies with this test, the labeling indicates that it meets *USP Drug Release Test 3*.**Medium:** 100 mM acetate buffer, pH 5.0, with 0.01% of cetyltrimethylammonium bromide (13.6 g/L of sodium acetate monohydrate in water, adjust with glacial acetic acid to a pH of 5.0, and add 0.1 g/L of cetyltrimethylammonium bromide); 900 mL**Apparatus 5:** 100 rpm, with the 76-mm disk**Times:** 8, 24, 96, and 168 h**Solution A:** 2.4 g/L of octanesulfonic acid sodium salt and 2 mL/L of phosphoric acid in water**Mobile phase:** Methanol and *Solution A* (45:55). Adjust with 10 N sodium hydroxide to a pH of 3.0.**Standard stock solution:** 1 mg/mL of USP Clonidine RS in methanol**Standard solution:** Dilute the *Standard stock solution* with *Medium* to obtain a final concentration similar to the expected concentration in the *Sample solution*, considering complete drug release.**Sample solution:** Apply double-sided adhesive tape to the stainless steel disk to cover enough of the disk area so that the entire patch is secured by the tape. Apply a Transdermal System with the release liner intact to the adhesive layer on the stainless steel disk. Press the backing film of the patch to the adhesive tape with the clear release liner film of the system facing up. Peel the release liner from the affixed system on the disk assembly, and place the disk assembly flat on the bottom of the vessel with the exposed transdermal adhesive side up and parallel to the bottom edge of the paddle blade. Lower the paddle, and start the equipment. At each sampling time withdraw an appropriate volume of the solution under test.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 220 nm**Column:** 4.6-mm × 15-cm; packing L7**Column temperature:** 30°**Flow rate:** 1.5 mL/min**Injection size:** 30 µL**System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.8**Relative standard deviation:** NMT 2.0%**Analysis:****Samples:** *Standard solution* and *Sample solution*Calculate the concentration (*C<sub>i</sub>*) of clonidine(C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>) in the *Medium* (mg/mL) at each time point:

$$C_i = (r_u/r_s) \times C_s$$

*r<sub>u</sub>* = peak response from the *Sample solution**r<sub>s</sub>* = peak response from the *Standard solution**C<sub>s</sub>* = concentration of the *Standard solution* (mg/mL)*i* = interval, where *i* = 1 at 8 h, *i* = 2 at 24 h, *i* = 3 at 96 h, *i* = 4 at 168 hCalculate the rate of clonidine (C<sub>9</sub>H<sub>9</sub>Cl<sub>2</sub>N<sub>3</sub>) released in µg/h/cm<sup>2</sup> at each time point:

$$\text{Result} = [(C_i - C_{i-1}) \times V_i \times 1000]/[S \times (T_i - T_{i-1})]$$

$$V_i = V_0 - [(i - 1) \times V_A]$$

*V<sub>i</sub>* = volume of *Medium* at a given time point*V<sub>0</sub>* = initial volume of *Medium*, 900 mL*V<sub>A</sub>* = volume of *Medium* withdrawn at each time point

1000 = conversion factor from mg to µg

*S* = system size in cm<sup>2</sup>*T<sub>i</sub>* = current time point*T<sub>i-1</sub>* = previous time point**Tolerances:** See *Table 4*.



Table 4

Time (h)	Release Rate ( $\mu\text{g}/\text{h}/\text{cm}^2$ )
8	6.5–11.0
24	2.5–5.5
96	2.5–5.0
168	2.0–3.8

The release rate of clonidine ( $\text{C}_9\text{H}_9\text{Cl}_2\text{N}_3$ ) from the Transdermal System, expressed as  $\mu\text{g}/\text{h}/\text{cm}^2$  at the times specified, conforms to *Acceptance Table 1* in (724).

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

**Standard stock solution:** 1 mg/mL of USP Clonidine Related Compound B RS in tetrahydrofuran

**Standard solutions:** Prepare a minimum of four *Standard solutions* in *Diluent* that bracket the expected clonidine related compound B concentration in the sample. The standard concentrations should be within the range of 0.2–10.0  $\mu\text{g}/\text{mL}$ .

[NOTE—The *Standard solutions* are stable for up to 2 days if stored at 4°.]

##### Analysis

**Samples:** At least three *Standard solutions* that will bracket the expected sample concentration range and the *Sample solution*

Measure the responses for clonidine related compound B. Calculate the peak response ratios of the analyte, and plot the results. Determine the linear regression equation of the standards by the mean-square method, and record the linear regression equation and the correlation coefficient: it should be NLT 0.995. Determine the concentration of clonidine related compound B.

Calculate the amount, in  $\mu\text{g}/\text{cm}^2$ , of clonidine related compound B in the portion of the Transdermal System taken:

$$\text{Result} = \text{CV}/A$$

C = concentration of clonidine related compound B from the linear regression analysis ( $\mu\text{g}/\text{mL}$ )

V = volume of the *Sample solution* (mL)

A = area of the sample system ( $\text{cm}^2$ )

Acceptance criteria: NMT 10.0  $\mu\text{g}/\text{cm}^2$

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in sealed, single-dose containers at a temperature not exceeding 30°.

- **LABELING:** The label states the total amount of clonidine in the Transdermal System and the release rate, in mg/day, for the duration of the application of one system. When more than one *Drug Release* test is given, the labeling states the *Drug Release* test used only if *Test 1* is not used.

##### • USP REFERENCE STANDARDS (11)

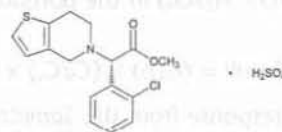
USP Clonidine RS

USP Clonidine Related Compound B RS

2-[(E)-2,6-Dichlorophenylimino]-1-(1-{2-[(E)-2,6-dichlorophenylimino]-imidazolidin-1-yl}-ethyl)imidazolidine.

$\text{C}_{20}\text{H}_{20}\text{Cl}_4\text{N}_6$  486.23

## Clopidogrel Bisulfate



$\text{C}_{16}\text{H}_{16}\text{ClNO}_2\text{S} \cdot \text{H}_2\text{SO}_4$  419.90  
Thieno[3,2-c]pyridine-5(4H)-acetic acid,  $\alpha$ -(2-chlorophenyl)-6,7-dihydro-, methyl ester, (S)-, sulfate (1:1);  
Methyl (+)-(S)- $\alpha$ -(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate, sulfate (1:1) [120202-66-6].

#### DEFINITION

Clopidogrel Bisulfate contains NLT 97.0% and NMT 101.5% of clopidogrel bisulfate ( $\text{C}_{16}\text{H}_{16}\text{ClNO}_2\text{S} \cdot \text{H}_2\text{SO}_4$ ), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (17K):**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **C. IDENTIFICATION TESTS—GENERAL, Sulfate (191):** Meets the requirements

#### ASSAY

##### • PROCEDURE

[NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]

**Buffer:** 1.36 g/L of monobasic potassium phosphate in water

**Mobile phase:** Acetonitrile and *Buffer* (25:75)

**System suitability stock solution:** 100  $\mu\text{g}/\text{mL}$  of USP Clopidogrel Bisulfate RS and 200  $\mu\text{g}/\text{mL}$  of USP Clopidogrel Related Compound B RS in methanol

**System suitability solution:** 2.5  $\mu\text{g}/\text{mL}$  of USP

Clopidogrel Bisulfate RS and 5.0  $\mu\text{g}/\text{mL}$  of USP

Clopidogrel Related Compound B RS in *Mobile phase* from *System suitability stock solution*

**Standard stock solution:** 1.0 mg/mL of USP

Clopidogrel Bisulfate RS in methanol

**Standard solution:** 0.1 mg/mL in *Mobile phase* from the *Standard stock solution*

**Sample stock solution:** 1 mg/mL of Clopidogrel Bisulfate in methanol

**Sample solution:** 0.1 mg/mL in *Mobile phase*, from *Sample stock solution*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  15-cm; packing L57

**Flow rate:** 1 mL/min

**Injection volume:** 10  $\mu\text{L}$

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for the two enantiomers of clopidogrel related compound B and for clopidogrel are 0.8, 1.2, and 1.0, respectively.]

**Suitability requirements**

**Resolution:** Greater than 2.5 between clopidogrel and the first enantiomer of clopidogrel related compound B, *System suitability solution*

**Relative standard deviation:** NMT 1.0% from clopidogrel bisulfate, *Standard solution*



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of clopidogrel bisulfate ( $C_{16}H_{16}ClNO_2S \cdot H_2SO_4$ ) in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of *Sample solution* (mg/mL)

Acceptance criteria: 97.0%–101.5% on the dried basis

**IMPURITIES**

• **RESIDUE ON IGNITION (281):** NMT 0.1%

• **ORGANIC IMPURITIES**

[NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]

**Buffer:** 0.96 g/L sodium 1-pentanesulfonate. Adjust with phosphoric acid to a pH of 2.5.

**Solution A:** Acetonitrile

**Solution B:** Methanol

**Mobile phase:** See Table 1.

Table 1

Time (min)	Buffer (%)	Solution A (%)	Solution B (%)
0	85	10	5
3	85	10	5
48	30	65	5
68	30	65	5

**Diluent:** Acetonitrile and Buffer (60:40)

**System suitability solution:** 6.5 mg/mL of USP

Clopidogrel Bisulfate RS and 0.01 mg/mL each of USP

Clopidogrel Related Compound A RS and USP

Clopidogrel Related Compound B RS in *Diluent*

**Standard solution:** 6.5 µg/mL of USP Clopidogrel Bisulfate RS in *Diluent*

**Sample solution:** 6.5 mg/mL of Clopidogrel Bisulfate in *Diluent*

**Chromatographic system**

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 3.9-mm × 15-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

**System suitability**

**Sample:** *System suitability solution*

[NOTE—The relative retention times for clopidogrel related compound A, clopidogrel, and clopidogrel related compound B are given in Table 2.]

**Suitability requirements**

**Peak-to-valley ratio ( $H_p/H_v$ ):** NLT 10 where  $H_p$  is the height above the baseline of the peak due to clopidogrel related compound B and  $H_v$  is the height above the baseline of the lowest point of the curve separating clopidogrel related compound B and clopidogrel, *System suitability solution*.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clopidogrel related compound A, clopidogrel related compound B, and any other individual impurity in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clopidogrel related compound A, clopidogrel related compound B, or any other impurity from the *Sample solution*

$r_S$  = peak response of clopidogrel from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Clopidogrel related compound A <sup>a</sup>	0.4	0.2
Clopidogrel	1.0	—
Clopidogrel related compound B <sup>b</sup>	1.1	0.3
Any other impurity <sup>c</sup>	—	0.10
Total impurities	—	0.5

<sup>a</sup> (+)-(5)-(o-Chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid.

<sup>b</sup> Methyl (+/-)-(o-chlorophenyl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-acetate.

<sup>c</sup> Disregard any peak less than 0.05%.

• **LIMIT OF CLOPIDOGREL RELATED COMPOUND C**

**Mobile phase:** Heptane and dehydrated alcohol (85:15)

**Standard solution:** 0.02 mg/mL each of USP

Clopidogrel Bisulfate RS, USP Clopidogrel Related Compound B RS, and USP Clopidogrel Related Compound C

RS prepared as follows. Dissolve a quantity of USP

Clopidogrel Bisulfate RS, USP Clopidogrel Related Compound B RS, and USP Clopidogrel Related Compound C

RS in dehydrated alcohol (about 50% of the volume of the flask), and dilute with heptane to volume.

**Sample solution:** 2 mg/mL of Clopidogrel Bisulfate prepared as follows. Transfer 100 mg of Clopidogrel Bisulfate to a 50-mL volumetric flask, dissolve in 25 mL of dehydrated alcohol, and dilute with heptane to volume.

**Chromatographic system**

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 10-µm packing L80

**Flow rate:** 0.8 mL/min

**Injection volume:** 10 µL

**Run time:** 1.25 times the retention time of clopidogrel

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for clopidogrel related compound B, clopidogrel, and clopidogrel related compound C are 0.7, 1.0, and 0.6, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between clopidogrel related compound C and clopidogrel related compound B

**Signal-to-noise ratio:** NLT 20 for clopidogrel related compound C peak

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clopidogrel related compound C in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clopidogrel related compound C from *Sample solution*

$r_S$  = peak response of clopidogrel related compound C from *Standard solution*



$C_s$  = concentration of the clopidogrel related compound C in *Standard solution* (mg/mL)  
 $C_u$  = concentration of Clopidogrel Bisulfate in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 0.5%

### SPECIFIC TESTS

#### • LOSS ON DRYING (731)

Analysis: Dry a sample at 105° for 2 h.

Acceptance criteria: NMT 0.5%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers and store at controlled room temperature.

#### • USP REFERENCE STANDARDS (11)

USP Clopidogrel Bisulfate RS

USP Clopidogrel Related Compound A RS

(+)-(S)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid, hydrochloride.

$C_{15}H_{14}ClNO_2S \cdot HCl$  344.26

USP Clopidogrel Related Compound B RS

Methyl (+/-)-(o-chlorophenyl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-acetate, hydrochloride.

$C_{16}H_{17}Cl_2NO_2S$  358.28

USP Clopidogrel Related Compound C RS

Methyl (-)-(R)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate, hydrogen sulfate.

$C_{16}H_{16}ClNO_2S \cdot H_2SO_4$  419.90

*Diluent* to volume. Mix well, centrifuge a portion of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

**Sample solution:** Shake each bottle of Oral Suspension thoroughly. Transfer 2.0 mL of the Oral Suspension to a 1-L volumetric flask, and dilute with *Diluent* to volume. Mix well, centrifuge a portion of the solution for 5 min at 14,000 rpm, and use the supernatant. Protect from light, and store at 2°–8°.

### Chromatographic system

(See Chromatography (621), *System Suitability*.)

Mode: LC

Detector: UV 235 nm

Column: 2.1-mm × 25-cm; 5-μm packing L7

Temperatures

Column: 35°

Autosampler: 5°

Flow rate: 0.3 mL/min

Injection volume: 20 μL

### System suitability

Sample: *Standard solution*

[NOTE—The retention time for clopidogrel is about 7.4 min.]

### Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0% for replicate injections

### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clopidogrel ( $C_{16}H_{16}ClNO_2S$ ) in the portion of Oral Suspension taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response of clopidogrel from the *Sample solution*

$r_s$  = peak response of clopidogrel from the *Standard solution*

$C_s$  = concentration of clopidogrel in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of clopidogrel in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

### SPECIFIC TESTS

- **PH (791):** 2.1–3.1

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at 2°–8° or at controlled room temperature.
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored at 2°–8° or at controlled room temperature
- **USP REFERENCE STANDARDS (11)**  
USP Clopidogrel Bisulfate RS

## Clopidogrel Compounded Oral Suspension

### DEFINITION

Clopidogrel Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of clopidogrel ( $C_{16}H_{16}ClNO_2S$ ).

Prepare Clopidogrel Compounded Oral Suspension 5 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Clopidogrel tablet(s) <sup>a</sup> equivalent to	525 mg
Vehicle: A 1:1 mixture of Ora-Plus <sup>b</sup> and Ora-Sweet <sup>b</sup> , a sufficient quantity to make	105 mL

<sup>a</sup> Clopidogrel 75-mg tablets, Dr. Reddy's Laboratory Limited, Bridgewater, NJ.

<sup>b</sup> Perrigo Pharmaceuticals, Allegan, MI.

Crush the *Clopidogrel tablet(s)* to a fine powder using a mortar and pestle or by other mechanical means. Wet the powder with a small amount of *Vehicle*, and triturate to make a smooth paste. Add the *Vehicle* to make the contents pourable. Transfer the contents stepwise and quantitatively to a calibrated container using the remainder of the *Vehicle*. Add sufficient *Vehicle* to bring to final volume. Shake to mix well.

### ASSAY

#### • PROCEDURE

**Solution A:** 10 mM sodium phosphate adjusted with phosphoric acid to a pH of 3.0. Pass through a nylon filter of 0.45-μm pore size, and degas.

**Mobile phase:** Acetonitrile and *Solution A* (65:35)

**Diluent:** Water adjusted with phosphoric acid to a pH of 3.0

**Standard stock solution:** 5 mg/mL of clopidogrel prepared from USP Clopidogrel Bisulfate RS and *Diluent*. Mix well, and sonicate for 3 min. Store at 2°–8°.

**Standard solution:** Transfer 2.0 mL of the *Standard stock solution* to a 1-L volumetric flask, and dilute with

## Clopidogrel Tablets

» Clopidogrel Tablets contain Clopidogrel Bisulfate equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of clopidogrel ( $C_{16}H_{16}ClNO_2S$ ).

**Packaging and storage**—Preserve in well-closed containers, and store at controlled room temperature.



**USP Reference standards (11)—**

USP Clopidogrel Bisulfate RS

USP Clopidogrel Related Compound A RS

(+)-(S)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid.

USP Clopidogrel Related Compound B RS

Methyl (±)-(o-chlorophenyl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-acetate, hydrochloride.

USP Clopidogrel Related Compound C RS

Methyl (–)-(R)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate, hydrogen sulfate.

**Identification—****A: Ultraviolet Absorption (197U)—***Spectral range:* 250 to 300 nm.*Solution*—Use the test solution prepared as directed in the test for *Uniformity of dosage units*.**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.**Dissolution (711)—***Medium:* pH 2.0 hydrochloric acid buffer (see *Buffer Solutions under Reagents, Indicators, and Solutions*); 1000 mL.*Apparatus 2:* 50 rpm.*Time:* 30 minutes.*Standard solution*—Dissolve an accurately weighed quantity of USP Clopidogrel Bisulfate RS in 20.0 mL of methanol, and dilute quantitatively, and stepwise if necessary, with *Medium* to obtain a solution having a known concentration corresponding to that of the solution under test.*Procedure*—Determine the amount of  $C_{16}H_{16}ClNO_2S$  dissolved by employing UV absorption at a wavelength of about 240 nm on filtered portions of the solution under test in comparison with the *Standard solution*.*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_{16}H_{16}ClNO_2S$  is dissolved in 30 minutes.**Uniformity of dosage units (905):** meet the requirements.*Procedure for content uniformity*—Using a suitable volumetric flask, place 1 Tablet in 50.0 mL of 0.1 N hydrochloric acid. Sonicate for 5 minutes, and cool. Quantitatively transfer 5.0 mL of this solution to the flask, and dilute with 0.1 N hydrochloric acid to 50.0 mL. Pass a portion of the solution through a suitable filter having a 0.45- $\mu$ m or finer porosity, discarding the first 5 mL of the filtrate. Determine the amount of clopidogrel by employing UV absorption at the wavelength of maximum absorbance at about 270 nm, in comparison with a *Standard solution* having a known concentration of USP Clopidogrel Bisulfate RS in 0.1 N hydrochloric acid.**Related compounds**—[NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]*Phosphate buffer and Mobile phase*—Prepare as directed in the *Assay under Clopidogrel Bisulfate*.*System suitability solution*—Dissolve accurately weighed quantities of USP Clopidogrel Bisulfate RS and USP Clopidogrel Related Compound B RS in methanol, and dilute with methanol to obtain a solution having concentrations of about 100  $\mu$ g per mL and 200  $\mu$ g per mL, respectively. Transfer 5 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.*Standard solution*—Dissolve accurately weighed quantities of USP Clopidogrel Bisulfate RS, USP Clopidogrel Related Compound A RS, and USP Clopidogrel Related Compound C RS in methanol to obtain a solution having known concentrations of about 40  $\mu$ g per mL, 250  $\mu$ g per mL, and 300  $\mu$ g per mL, respectively. Transfer 5 mL of this solution to a 200-mL volumetric flask, and dilute with *Mobile phase*to volume. This solution contains about 1  $\mu$ g of clopidogrel bisulfate per mL, 6  $\mu$ g of clopidogrel related compound A per mL, and 7.5  $\mu$ g of clopidogrel related compound C per mL.*Test solution*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of clopidogrel (free base), to a 200-mL volumetric flask, add 5 mL of methanol, dilute with *Mobile phase* to volume, and mix. Allow to stand for 10 minutes, and mix. Pass a portion of this solution through a filter having a 0.45- $\mu$ m or finer porosity, and use the filtrate after discarding the first 5 mL.*Chromatographic system* (see *Chromatography (621)*)—The liquid chromatograph is equipped with a 220-nm detector and 4.6-mm  $\times$  15-cm column that contains packing L57. The flow rate is about 1.0 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.8 and 1.2 for the two enantiomers of clopidogrel related compound B and 1.0 for clopidogrel; and the resolution,  $R$ , between clopidogrel and the first enantiomer of clopidogrel related compound B is greater than 2.5. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.5 for clopidogrel related compound A, 1.0 for clopidogrel and 2.0 for clopidogrel related compound C; and the relative standard deviation for replicate injections is not more than 15% for each peak.*Procedure*—Inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of clopidogrel related compounds A and C in the portion of Tablets taken by the formula:

$$20(321.82/419.90)(C/W)(r_u/r_s)$$

in which 321.82 is the molecular weight of clopidogrel; 419.90 is the molecular weight of clopidogrel bisulfate; C is the concentration, in  $\mu$ g per mL, of the relevant clopidogrel related compound in the *Standard solution*; W is the weight, in mg, of clopidogrel in the portion of Tablets used to prepare the *Test solution* based on the labeled quantity of clopidogrel per Tablet, Tablet weight, and the weight of the portion of Tablets used; and  $r_u$  and  $r_s$  are the peak responses of the corresponding related compounds obtained from the *Test solution* and the *Standard solution*, respectively.

Calculate the percentage of any other impurity (excluding clopidogrel related compound B) in the portion of Tablets taken by the formula:

$$20(321.82/419.90)(C_c/W)(r_u/r_s)$$

in which  $C_c$  is the concentration of clopidogrel bisulfate, in  $\mu$ g per mL, in the *Standard solution*;  $r_u$  is the peak response of any other impurity obtained from the *Test solution*;  $r_s$  is the peak response of clopidogrel peak obtained from the *Standard solution*; and the other terms are as defined above: not more than 1.2% of clopidogrel related compound A is found, not more than 1.5% of clopidogrel related compound C is found, not more than 0.2% of any other single impurity (excluding clopidogrel related compound B) is found, and not more than 2.5% of total impurities (excluding clopidogrel related compound B) is found.**Assay**—[NOTE—For all clopidogrel related compounds, the concentrations are expressed as bisulfate salts. Use bisulfate salt equivalents stated on USP Reference Standards labels to calculate the concentrations as appropriate.]*Phosphate buffer, Mobile phase, and Chromatographic system*—Proceed as directed in the *Assay under Clopidogrel Bisulfate*.*System suitability preparation*—Dissolve accurately weighed quantities of USP Clopidogrel Bisulfate RS and USP Clopidogrel Related Compound B RS in methanol, and



quantitatively dilute with methanol to obtain a solution having concentrations of about 100 µg per mL and 200 µg per mL, respectively. Transfer 5 mL of this solution to a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Clopidogrel Bisulfate RS in methanol to obtain a solution having a known concentration of about 0.1 mg of clopidogrel bisulfate per mL.

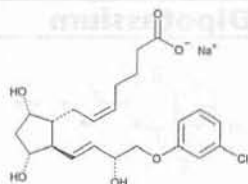
**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of clopidogrel (base), to a 100-mL volumetric flask, and add 50 mL of methanol. Sonicate for 5 minutes, and stir for 30 minutes. Dilute with methanol to volume, and mix. Transfer 5.0 mL of this solution to the flask, dilute with methanol to 50.0 mL, and mix. Pass a portion of this solution through a filter having a 0.45-µm or finer porosity, and use the filtrate after discarding the first 5 mL.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the analyte peaks. Calculate the quantity, in mg, of clopidogrel ( $C_{16}H_{16}ClNO_2S$ ) in the portion of Tablets taken by the formula:

$$1000(321.82/419.90)C(r_U / r_S)$$

in which 321.82 is the molecular weight of clopidogrel; 419.90 is the molecular weight of clopidogrel bisulfate; C is the concentration, in mg per mL, of USP Clopidogrel Bisulfate RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cloprostenol Sodium



$C_{22}H_{28}ClNaO_6$  446.90  
5-Heptenoic acid, 7-[2-[4-(3-chlorophenoxy)-3-hydroxy-1-butenyl]-3,5-dihydroxycyclopentyl]-, [1 $\alpha$ (Z),2 $\beta$ (1E,3R\*),3 $\alpha$ ,5 $\alpha$ ]-, sodium salt, ( $\pm$ )-;  
( $\pm$ )-Sodium (Z)-7-[(1R\*,2R\*,3R\*,5S\*)-2-[(E)-(3R\*)-4-(m-chlorophenoxy)-3-hydroxy-1-butenyl]-3,5-dihydroxycyclopentyl]-5-heptenoate [55028-72-3].

### DEFINITION

Cloprostenol Sodium contains NLT 97.5% and NMT 102.5% of cloprostenol sodium ( $C_{22}H_{28}ClNaO_6$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (17K)
- **B. IDENTIFICATION TESTS—GENERAL**, Sodium (19I)

### ASSAY

#### • PROCEDURE

**Mobile phase:** Chromatographic hexane, dehydrated alcohol, and glacial acetic acid (900:100:1)

**Standard solution:** 0.8 mg/mL of USP Cloprostenol Sodium RS in dehydrated alcohol

**Sample solution:** 0.8 mg/mL of Cloprostenol Sodium in dehydrated alcohol

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm; 5-µm packing L3

**Flow rate:** 1.8 mL/min

**Injection volume:** 5 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5 for the cloprostenol peak

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cloprostenol sodium ( $C_{22}H_{28}ClNaO_6$ ) in the portion of Cloprostenol Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cloprostenol Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cloprostenol Sodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.5%–102.5% on the anhydrous basis

### IMPURITIES

#### • ORGANIC IMPURITIES

**Mobile phase:** Chromatographic hexane, dehydrated alcohol, and glacial acetic acid (930:70:1)

**Sample solution:** 20 mg/mL of Cloprostenol Sodium in dehydrated alcohol

**Standard solution, Chromatographic system, and System suitability:** Proceed as directed in the Assay, except use a run time of NLT 2 times the retention time of cloprostenol.

### Analysis

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Cloprostenol Sodium taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_T$  = sum of the responses all the peaks from the *Sample solution*

**Acceptance criteria:** Disregard any peak below 0.05%.

**Individual impurities:** NMT 1.0%

**Total impurities:** NMT 2.5%

### SPECIFIC TESTS

#### • WATER DETERMINATION, Method I (921)

**Sample solution:** 50 mg dissolved in 1 mL of dehydrated alcohol

**Acceptance criteria:** NMT 3.0%

### ADDITIONAL REQUIREMENTS

- **LABELING:** Label it to indicate that it is for veterinary use only.
- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Cloprostenol Sodium RS

## Cloprostenol Injection

» Cloprostenol Injection is a sterile solution of Cloprostenol Sodium in Water for Injection. It



contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cloprostenol ( $C_{22}H_{29}ClO_6$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers protected from light. Store at controlled room temperature.

**Labeling**—Label it to indicate that it is for veterinary use only, and to indicate the strength as the equivalent amount of cloprostenol per dose.

**USP Reference standards** (11)—

USP Cloprostenol Sodium RS

USP Endotoxin RS

USP Hydrocortisone Acetate RS

**Identification**—

**A:** The retention time of the cloprostenol peak in the chromatogram of the *Assay preparation* corresponds to that of the cloprostenol peak in the chromatogram of the *Standard preparation*, obtained as directed in the *Assay*.

**B:** It meets the requirements of the test for *Sodium* (191).

**Bacterial Endotoxins Test** (85)—It contains not more than 2500 USP Endotoxin Units per mg of cloprostenol.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**Related compounds**—

*Mobile phase* and *System suitability solution*—Prepare as directed in the *Assay*.

*Standard solution*—Prepare as directed for *Standard preparation under Assay*.

*Test solution*—Prepare as directed for *Assay preparation*.

*Chromatographic system* (see *Chromatography* (621))—Prepare as directed in the *Assay*.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure all of the peak responses. Calculate the percentage of each impurity in the portion of Injection taken by the formula:

$$100(C_5 / C_7)(r_i / r_s)$$

in which  $C_5$  is the concentration, in mg per mL, of USP Cloprostenol Sodium RS in the *Standard solution*;  $C_7$  is the concentration, in mg per mL, of cloprostenol in the *Test solution*;  $r_i$  is the peak response for each impurity obtained from the *Test solution*; and  $r_s$  is the peak response of cloprostenol obtained from the *Standard solution*: not more than 1.0% of any individual impurity is found, and not more than 2.5% of total impurities is found. Disregard any peak below 0.05%.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—

*pH 2.5 Monobasic sodium phosphate solution*—Prepare an aqueous solution containing 2.4 mg of monobasic sodium phosphate dihydrate per mL of solution. Adjust with phosphoric acid to a pH of 2.5.

*Mobile phase*—Prepare a filtered and degassed mixture of *pH 2.5 Monobasic sodium phosphate solution* and acetonitrile (73:27). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*System suitability solution*—Dissolve an accurately weighed quantity of USP Cloprostenol Sodium RS and USP Hydrocortisone Acetate RS in dehydrated alcohol, and dilute with *Mobile phase* to obtain a solution containing about 0.25 mg of cloprostenol sodium and 0.5 mg of hydrocortisone acetate per mL of solution.

*Standard preparation*—Dissolve an accurately weighed quantity of USP Cloprostenol Sodium RS in dehydrated al-

cohol, and dilute quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution having a known concentration of about 0.1 mg per mL.

*Assay preparation*—Dilute a volume of Injection in dehydrated alcohol and dilute quantitatively, and stepwise if necessary, with dehydrated alcohol to obtain a solution containing 0.1 mg of cloprostenol per mL of solution, based on the label claim.

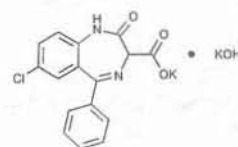
*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 5-mm  $\times$  25-cm column that contains packing L1. The flow rate is about 1.8 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between the hydrocortisone acetate peak and the cloprostenol peak is not less than 6. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the cloprostenol peak. Calculate the percentage label claim of cloprostenol ( $C_{22}H_{29}ClO_6$ ) in the portion of Injection taken by the formula:

$$100(C_5 / C_U)(r_U / r_S)(M_1 / M_2)$$

in which  $C_5$  is the concentration, in mg per mL, of USP Cloprostenol Sodium RS in the *Standard preparation*;  $C_U$  is the concentration, in mg per mL, of cloprostenol in the *Assay preparation*;  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively,  $M_1$  is the molecular weight of cloprostenol (424.92), and  $M_2$  is the molecular weight of cloprostenol sodium (446.90).

## Clorazepate Dipotassium



$C_{16}H_{11}ClK_2N_2O_4$  408.92

1H-1,4-Benzodiazepine-3-carboxylic acid, 7-chloro-2,3-dihydro-2-oxo-5-phenyl-, potassium salt compound with potassium hydroxide (1:1).

Potassium 7-chloro-2,3-dihydro-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-carboxylate compound with potassium hydroxide (1:1) [57109-90-7].

» Clorazepate Dipotassium contains not less than 98.5 percent and not more than 101.5 percent of  $C_{16}H_{11}ClK_2N_2O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve under nitrogen in tight, light-resistant containers.

**USP Reference standards** (11)—

USP 2-Amino-5-chlorobenzophenone RS

$C_{13}H_9ClNO$  231.68

USP Nordazepam RS

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one.

$C_{15}H_{11}ClN_2O$  270.72

USP Clorazepate Dipotassium RS



**Identification—**

**A: Infrared Absorption** (197M).

**B: Ultraviolet Absorption** (197U)—

*Solution:* 7 µg per mL.

*Medium:* sodium hydroxide solution (1 in 2500).

**Loss on drying** (731)—Dry it in vacuum at 60° for 1 hour: it loses not more than 0.5% of its weight.

**Delete the following:**

• **Heavy metals, Method II** (231): 0.002%. • (Official 1-Jan-2018)

**Related compounds—****TEST 1—**

*Phosphate buffer solution*—Dissolve about 13.8 g of monobasic sodium phosphate in 500 mL of water, adjust with 2.5 N sodium hydroxide to a pH of 8.0, and mix.

*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and *Phosphate buffer solution* (5:4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Internal standard solution*—Dissolve about 5 mL of 2,6-dimethylaniline in 50 mL of hexane, and carefully add dropwise hydrochloric acid to precipitate the amine hydrochloride. Filter through a sintered-glass funnel, wash the solid precipitate with hexane, and allow the precipitate to dry. Transfer about 50 mg of the dried precipitate of 2,6-dimethylaniline hydrochloride to a 100-mL volumetric flask, add 10.0 mL of *Phosphate buffer solution* and 40 mL of water, and dilute with acetonitrile to volume.

*Standard solution*—Dissolve an accurately weighed quantity of USP Nordazepam RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 75 µg per mL. Transfer 4.0 mL of this solution to a 50-mL conical flask, add 4.0 mL of 0.7 M potassium carbonate, 2.0 mL of *Internal standard solution*, and 15.0 mL of water. Insert a stopper, and mix.

*Test solution*—Transfer an accurately weighed quantity of about 50 mg of Clorazepate Dipotassium to a 50-mL conical flask. Add 4.0 mL of 0.7 M potassium carbonate, and start stirring the solution. Add 2 mL of *Internal standard solution* and 19.0 mL of water. Stop stirring about 5 minutes after the addition of the 0.7 M potassium carbonate solution. [NOTE—Prepare fresh immediately before each injection.]

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 232-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.0 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative retention time for 2,6-dimethylaniline is about 0.8 and 1.0 for nordazepam; the relative standard deviation of the peak area ratio of nordazepam to 2,6-dimethylaniline for replicate injections is not more than 2.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak areas. Calculate the percentage of nordazepam in the portion of Clorazepate Dipotassium taken by the formula:

$$2500(C/W) (R_i / R_s)$$

in which *C* is the concentration, in mg per mL, of USP Nordazepam RS in the *Standard solution*; *W* is the weight, in mg, of Clorazepate Dipotassium taken to prepare the *Test solution*; *R<sub>i</sub>* is the peak area ratio of any impurity to 2,6-dimethylaniline obtained from the *Test solution*; and *R<sub>s</sub>* is the peak area ratio of nordazepam to 2,6-dimethylaniline obtained from the *Standard solution*: not more than 0.5% of

nordazepam is found and not more than 0.1% of any individual impurity is found.

**TEST 2—**

*Diluent*—Prepare a mixture of 0.001 N sodium hydroxide and acetonitrile (1:1).

*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and a 1 M solution of tetrabutylammonium hydroxide in methanol (110:90:1), adjust with phosphoric acid to a pH of 7.7, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

*Standard solution*—Dissolve an accurately weighed quantity of USP 2-Amino-5-chlorobenzophenone RS in *Diluent*, and dilute quantitatively, and stepwise if necessary, with *Diluent*, to obtain a solution having a known concentration of about 0.0026 mg per mL.

*Test solution*—Transfer about 300 mg of Clorazepate Dipotassium, accurately weighed, to a glass test tube. Add 10.0 mL of *Diluent*, and vigorously mix on a vortex mixer for about 90 seconds. [NOTE—Prepare fresh immediately before each injection.]

*Chromatographic system* (see *Chromatography* (621))—The liquid chromatograph is equipped with a 238-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation of the peak height for replicate injections is not more than 3.0%.

*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of each impurity in the portion of Clorazepate Dipotassium taken by the formula:

$$1000(C/W)(r_i / r_s)$$

in which *C* is the concentration, in mg per mL, of USP 2-Amino-5-chlorobenzophenone RS in the *Standard solution*; *W* is the weight, in mg, of sample taken; *r<sub>i</sub>* is the peak height of each impurity obtained from the *Test solution*; and *r<sub>s</sub>* is the peak height of 2-amino-5-chlorobenzophenone obtained from the *Standard solution*: not more than 0.1% of 2-amino-5-chlorobenzophenone is found, not more than 0.1% of any other individual impurity is found, and not more than 1.0% of total impurities in *Test 1* and *Test 2* is found.

**Assay**—Transfer about 150 mg of Clorazepate Dipotassium, accurately weighed, to a 250-mL beaker, add 100 mL of glacial acetic acid, and stir until dissolved. Titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically, using a glass electrode and a calomel electrode containing a 1 in 100 solution of lithium perchlorate in glacial acetic acid. Perform a blank determination (see *Titrimetry* (541)), and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 13.63 mg of C<sub>16</sub>H<sub>11</sub>ClK<sub>2</sub>N<sub>2</sub>O<sub>4</sub>.

**Clorazepate Dipotassium Tablets**

» Clorazepate Dipotassium Tablets contain not less than 90.0 percent and not more than 110.0 percent of clorazepate dipotassium (C<sub>16</sub>H<sub>11</sub>ClK<sub>2</sub>N<sub>2</sub>O<sub>4</sub>).

**Packaging and storage**—Preserve in tight, light-resistant containers.



**USP Reference standards** (11)—

USP 2-Amino-5-chlorobenzophenone RS

 $C_{13}H_{10}ClNO$  231.68

USP Nordazepam RS

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one.

 $C_{15}H_{11}ClN_2O$  270.72

USP Clorazepate Dipotassium RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.**Dissolution** (711)—*Medium*: 0.01 N hydrochloric acid; 900 mL.*Apparatus 2*: 50 rpm.*Time*: 30 minutes.*Procedure*—Determine the amount of  $C_{16}H_{11}ClK_2N_2O_4$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 240 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a *Standard solution* having a known concentration of USP Clorazepate Dipotassium RS in the same *Medium*.*Tolerances*—Not less than 80% (Q) of the labeled amount of  $C_{16}H_{11}ClK_2N_2O_4$  is dissolved in 30 minutes.**Uniformity of dosage units** (905): meet the requirements.**PROCEDURE FOR CONTENT UNIFORMITY**—*Standard solution*—Dissolve an accurately weighed quantity of USP Clorazepate Dipotassium RS in 0.01 M sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with 0.01 M sodium hydroxide to obtain a solution having a known concentration of about 7.6 µg per mL.*Test solution*—Transfer 1 Tablet to a suitable container, add 200 mL of 0.01 M sodium hydroxide, and homogenize for not less than 3 minutes. Centrifuge a portion of this solution for 15 minutes, and filter the supernatant, discarding the first 20 mL. Dilute an accurately measured portion of the filtrate with 0.01 M sodium hydroxide to obtain a solution having a known concentration of about 7.6 µg per mL.*Procedure*—Concomitantly determine the absorbances of the *Standard solution* and the *Test solution* in 1-cm cells at the wavelength of maximum absorbance at about 231 nm, with a suitable spectrophotometer, using 0.01 M sodium hydroxide as the blank.**Related compounds**—**METHOD I**—*Phosphate buffer solution*—Dissolve about 13.8 g of monobasic sodium phosphate in 500 mL of water, adjust with 1 N sodium hydroxide to a pH of 8.0, and mix.*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and *Phosphate buffer solution* (5:4:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).*Standard solution*—Dissolve an accurately weighed quantity of USP Nordazepam RS in acetonitrile, and dilute quantitatively, and stepwise if necessary, with acetonitrile to obtain a solution having a known concentration of about 66 µg per mL. Transfer 4.0 mL of this solution to a 25-mL volumetric flask, add 5.0 mL of 0.7 M potassium carbonate and 3.0 mL of acetonitrile, dilute with water to volume, mix, and filter.*Test solution*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 15 mg of clorazepate dipotassium, to a suitable container. Add 5 mL of acetonitrile, 5 mL of 0.7 M potassium carbonate, and 15 mL of water, stir for 10 minutes, and filter. [NOTE—Prepare fresh before each injection, and use within 3 minutes.]*Chromatographic system*—The liquid chromatograph is equipped with a 232-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms for not less than twice the retention time of nordazepam, and measure the peak responses. Calculate the quantity, in mg, of each impurity in the portion of Tablets taken by the formula:

$$25C(r_i / r_s)$$

in which C is the concentration, in mg per mL, of USP Nordazepam RS in the *Standard solution*;  $r_i$  is the peak response of each impurity obtained from the *Test solution*; and  $r_s$  is the peak response for nordazepam obtained from the *Standard solution*: not more than 2.0% of nordazepam is found.**METHOD II**—*Mobile phase*—Prepare a filtered and degassed mixture of water, acetonitrile, and a 1 M solution of tetrabutylammonium hydroxide in methanol (110:90:1), adjust with phosphoric acid to a pH of 7.7, and mix. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).*Standard solution*—Dissolve an accurately weighed quantity of USP 2-Amino-5-chlorobenzophenone RS in acetonitrile to obtain a solution having a known concentration of about 0.50 mg per mL. Dilute with water to obtain a solution having a known concentration of about 0.25 mg per mL. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with a mixture of 0.1 mM sodium hydroxide and acetonitrile (7:3) to volume, and mix. Transfer 15 mL of this solution to a 50-mL volumetric flask, dilute with a mixture of 0.1 mM sodium hydroxide and acetonitrile (7:3) to volume, and mix.*Test solution*—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 15 mg of clorazepate dipotassium, to a suitable container, add 10 mL of a mixture of 0.1 mM sodium hydroxide and acetonitrile (7:3), mix, shake by mechanical means for 10 minutes, and filter.*Chromatographic system*—The liquid chromatograph is equipped with a 238-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.*Procedure*—Separately inject equal volumes (about 20 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the quantity, in mg, of each impurity in the portion of Tablets taken by the formula:

$$10C(r_i / r_s)$$

in which C is the concentration, in mg per mL, of USP 2-Amino-5-chlorobenzophenone RS in the *Standard solution*;  $r_i$  is the peak response of each impurity obtained from the *Test solution*; and  $r_s$  is the response of the 2-amino-5-chlorobenzophenone peak obtained from the *Standard solution*: the sum of all impurities, other than nordazepam, found in *Method I* and *Method II* is not more than 0.5%.**Assay**—*Buffer solution*—Transfer 5.0 mL of 1 M tetrabutylammonium hydroxide in methanol to a 1-L volumetric flask, dilute with water to volume, adjust with phosphoric acid to a pH of 7.5, and mix.



**Mobile phase**—Prepare a filtered and degassed mixture of *Buffer solution* and acetonitrile (7:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Clorazepate Dipotassium RS in 0.01 M sodium hydroxide, and dilute quantitatively, and stepwise if necessary, with 0.01 M sodium hydroxide to obtain a solution having a known concentration of about 60 µg per mL. Shake by mechanical means for 15 minutes, and filter.

**Assay preparation**—Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 75 mg of clorazepate dipotassium, to a suitable container, add 200 mL of 0.01 M sodium hydroxide, and homogenize for not less than 3 minutes. Transfer 15 mL of this solution to a 100-mL volumetric flask, dilute with 0.01 M sodium hydroxide to volume, mix, and filter.

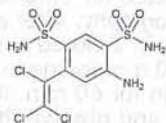
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 230-nm detector and a 3.9-mm × 30-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses of the major peaks. Calculate the quantity, in mg, of clorazepate dipotassium (C<sub>16</sub>H<sub>11</sub>ClK<sub>2</sub>N<sub>2</sub>O<sub>4</sub>) in the portion of Tablets taken by the formula:

$$1333C(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Clorazepate Dipotassium RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Clorsulon



C<sub>8</sub>H<sub>8</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub> 380.66  
1,3-Benzenedisulfonamide, 4-amino-6-(trichloroethenyl)-;  
4-Amino-6-(trichlorovinyl)-*m*-benzenedisulfonamide  
[60200-06-8].

### DEFINITION

Clorsulon contains NLT 98.0% and NMT 101.0% of clorsulon (C<sub>8</sub>H<sub>8</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub>), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197M)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

[NOTE—Store the *Standard solution* and the *Sample solution* in low-actinic glassware.]

**Mobile phase:** Acetonitrile, glacial acetic acid, and water (30:0.1:70)

**Standard solution:** 0.1 mg/mL of USP Clorsulon RS in *Mobile phase*

**Sample stock solution:** 1 mg/mL of Clorsulon in *Mobile phase*

**Sample solution:** 0.1 mg/mL of Clorsulon in *Mobile phase*, from the *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 30 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Column efficiency:** NLT 7400 theoretical plates

**Tailing factor:** NMT 1.4

**Relative standard deviation:** NMT 1.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clorsulon (C<sub>8</sub>H<sub>8</sub>Cl<sub>3</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub>) in the portion of Clorsulon taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Clorsulon RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Clorsulon in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–101.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

#### Delete the following:

- **HEAVY METALS, Method II** (231): NMT 30 ppm (Official 1-Jan-2018)

#### ORGANIC IMPURITIES

[NOTE—Store the *Standard solutions* and the *Sample solution* in low-actinic glassware.]

**Standard solution A:** 10 mg/mL of USP Clorsulon RS in methanol

**Standard solution B:** 0.1 mg/mL of USP Clorsulon RS in methanol, from *Standard solution A*

**Sample solution:** 10 mg/mL of Clorsulon in methanol

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Developing solvent system:** Chloroform and methanol (4:1)

#### Analysis 1

**Samples:** *Standard solution A*, *Standard solution B*, and *Sample solution*

Apply 10 µL each of the *Sample solution* and *Standard solution A*, and 5 and 10 µL of *Standard solution B*. Allow the spots to dry. Develop in *Developing solvent system* until the solvent front has moved three-fourths of the length of the plate. Remove the plate, mark the solvent front, allow the solvent to evaporate, and examine the plate under short-wavelength UV light.

**Acceptance criteria 1:** The chromatograms show principal spots at the same  $R_f$  value.

**Analysis 2:** Estimate the amounts of any additional spots observed in the chromatograms of the *Sample solution* in *Analysis 1* by comparing them with the spots in the two chromatograms of *Standard solution B*, corresponding to 0.5% and 1.0% of impurities.



**Acceptance criteria 2**

Any individual impurities: 0.5%; no spot other than the principal spot of the *Sample solution* is larger or more intense than that of the principal spot of the 5- $\mu$ L portion of *Standard solution B*.

Total impurities: NMT 2.0%

**SPECIFIC TESTS**

• **MELTING RANGE** (741): 197°–203°

• **LOSS ON DRYING** (731)

Analysis: Dry a sample under vacuum at 100° for 4 h.

Acceptance criteria: NMT 0.5%

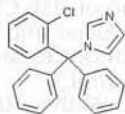
**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **LABELING:** Label it to indicate that it is for veterinary use only.

• **USP REFERENCE STANDARDS** (11)

USP Clorsulon RS

**Clotrimazole**

$C_{22}H_{17}ClN_2$  344.84

1*H*-Imidazole, 1-[(2-chlorophenyl)diphenylmethyl]-; 1-(*o*-Chloro- $\alpha,\alpha$ -diphenylbenzyl)imidazole [23593-75-1].

**DEFINITION**

Clotrimazole contains NLT 98.0% and NMT 102.0% of  $C_{22}H_{17}ClN_2$ , calculated on the dried basis.

**IDENTIFICATION**

• **A. INFRARED ABSORPTION** (197M)

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 4.35 mg/mL of dibasic potassium phosphate  
**Mobile phase:** Acetonitrile and *Buffer* (3:1). Pass through a membrane filter having a 0.2- $\mu$ m or finer pore size. The ratio of volumes may be changed to obtain the required resolution.

**Standard solution:** 0.5 mg/mL of USP Clotrimazole RS in methanol

**System suitability solution:** 0.1 mg/mL each of USP Clotrimazole RS and USP Clotrimazole Related Compound A RS in methanol

**Sample solution:** 0.5 mg/mL of Clotrimazole in methanol

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 25  $\mu$ L

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clotrimazole and clotrimazole related compound A are 1.0 and 1.2, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between clotrimazole and clotrimazole related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{22}H_{17}ClN_2$  in the portion of Clotrimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clotrimazole from the *Sample solution*

$r_S$  = peak response of clotrimazole from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

**IMPURITIES****Inorganic Impurities**

• **RESIDUE ON IGNITION** (281): NMT 0.1%

**Delete the following:**

• **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1-Jan-2018)

**Organic Impurities**• **PROCEDURE 1: LIMIT OF IMIDAZOLE**

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Standard solution:** 500  $\mu$ g/mL of USP Imidazole RS in chloroform

**Sample solution:** 100 mg/mL of Clotrimazole in chloroform

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Methanol and chloroform (3:2)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Proceed as directed for *Chromatography* (621), *Thin-Layer Chromatography*. After air-drying the plate for 5 min, place it in a closed container with a dish containing 100 g of iodine in a shallow layer, and allow to remain for 60 min. Remove the plate from the container, and observe the chromatogram.

**Acceptance criteria:** Any brown spot from the *Sample solution* at an  $R_f$  value corresponding to the principal spot from the *Standard solution* is not greater in size or intensity than the principal spot from the *Standard solution*: NMT 0.5% of imidazole.

• **PROCEDURE 2: LIMIT OF CLOTRIMAZOLE RELATED COMPOUND A**

**Buffer, Mobile phase, System suitability solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 50  $\mu$ g/mL of USP Clotrimazole Related Compound A RS prepared by dissolving in methanol using about 75% of the final flask volume. Dilute with *Buffer* to volume.

**Sample solution:** Transfer 100 mg of Clotrimazole to a 10-mL volumetric flask, add 5 mL of methanol to dissolve, add 2.5 mL of *Buffer*, dilute with methanol to volume, and mix.

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clotrimazole related compound A in the portion of Clotrimazole taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response of clotrimazole related compound A from the *Sample solution*  
 $r_S$  = peak response of clotrimazole related compound A from the *Standard solution*  
 $C_S$  = concentration of the *Standard solution* (mg/mL)  
 $C_U$  = concentration of the *Sample solution* (mg/mL)  
 Acceptance criteria: NMT 0.5%

**SPECIFIC TESTS**

- **LOSS ON DRYING** (731): Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)
  - USP Clotrimazole RS
  - USP Clotrimazole Related Compound A RS (o-Chlorophenyl)diphenylmethanol.
  - $C_{19}H_{15}ClO$  294.78
  - USP Imidazole RS

**Clotrimazole Cream****DEFINITION**

Clotrimazole Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ).

**IDENTIFICATION**

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 4.35 mg/mL of dibasic potassium phosphate

**Mobile phase:** Acetonitrile and *Buffer* (3:1)

[NOTE—The ratio of volumes may be changed to obtain the required resolution.]

**Standard solution:** 0.5 mg/mL of USP Clotrimazole RS in methanol

**System suitability solution:** 0.1 mg/mL each of USP Clotrimazole RS and USP Clotrimazole Related Compound A RS in methanol

**Sample solution:** Transfer the equivalent of 25 mg of clotrimazole from the Cream to a 50-mL screw-capped centrifuge tube. Add 25.0 mL of methanol, and heat at 50° in a water bath for 5 min, with occasional shaking. Remove the tube from the bath, and shake vigorously for 5 min. Cool in a methanol-ice bath for 15 min, and promptly centrifuge. Transfer the supernatant to a 50-mL volumetric flask. Add 20.0 mL of methanol to the residue in the centrifuge tube, and repeat the extraction starting with "heat at 50° in a water bath". Transfer the supernatant to the volumetric flask containing the supernatant from the first extraction, dilute with methanol to volume, and mix.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 25 μL

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for clotrimazole and clotrimazole related compound A are 1.0 and 1.2, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between clotrimazole and clotrimazole related compound A, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_{22}H_{17}ClN_2$  in the portion of Cream taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of clotrimazole from the *Sample solution*  
 $r_S$  = peak response of clotrimazole from the *Standard solution*  
 $C_S$  = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)  
 Acceptance criteria: 90.0%–110.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers, at a temperature between 2° and 30°.
- **LABELING:** Cream that is packaged and labeled for use as a vaginal preparation shall be labeled Clotrimazole Vaginal Cream.
- **USP REFERENCE STANDARDS** (11)
  - USP Clotrimazole RS
  - USP Clotrimazole Related Compound A RS (o-Chlorophenyl)diphenylmethanol.
  - $C_{19}H_{15}ClO$  294.78

**Clotrimazole Lotion****DEFINITION**

Clotrimazole Lotion contains NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak for clotrimazole of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**• **PROCEDURE**

**Buffer:** 4.35 g/L of dibasic potassium phosphate in water

**Mobile phase:** Methanol and *Buffer* (3:1). Pass through a filter of 0.5-μm or finer pore size.

**Internal standard solution:** 0.07 mg/mL of testosterone propionate in dehydrated alcohol

**Standard stock solution A:** 2 mg/mL of USP Clotrimazole RS in dehydrated alcohol

**Standard stock solution B:** 0.1 mg/mL of USP Clotrimazole Related Compound A RS in dehydrated alcohol

**Standard solution:** *Standard stock solution A*, *Standard stock solution B*, and *Internal standard solution* (5.0: 5.0: 10.0)

**Sample solution:** Nominally 1 mg/mL, prepared as follows. Transfer the equivalent of 10 mg of clotrimazole from freshly mixed Lotion to a screw-capped, 50-mL centrifuge tube. Add 10.0 mL of *Internal standard solution*, place the cap on the tube, and heat at 50° in a water bath for 5 min, with occasional shaking. Remove the tube from the bath, and shake vigorously for 5 min. Cool in a methanol-ice bath for 15 min, and promptly centrifuge. Transfer the supernatant to a test tube. Add 10.0 mL of dehydrated alcohol to the residue in the



centrifuge tube, and repeat the extraction as directed above, beginning with "place the cap on the tube". Transfer the supernatant to the test tube containing the supernatant from the first extraction.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 254 nm

#### Columns

Guard: 2.1-mm × 6-cm, 10-μm packing L2

Analytical: 3.9-mm × 30-cm; 10-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

#### System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for clotrimazole related compound A, clotrimazole, and testosterone propionate are 0.9, 1.0, and 1.5, respectively.]

#### Suitability requirements

Resolution: NLT 1.2 between clotrimazole related compound A and clotrimazole, and NLT 1.9 between clotrimazole and testosterone propionate

Relative standard deviation: NMT 2.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount clotrimazole (C<sub>22</sub>H<sub>17</sub>ClN<sub>2</sub>) in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of clotrimazole to testosterone propionate from the *Sample solution*

$R_S$  = peak response ratio of clotrimazole to testosterone propionate from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### IMPURITIES

##### • ORGANIC IMPURITIES: LIMIT OF CLOTRIMAZOLE RELATED COMPOUND A

Analysis: Using the chromatograms of the *Standard solution* and *Sample solution* as obtained in the Assay, calculate the percentage of clotrimazole related compound A in the portion of Lotion taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of clotrimazole related compound A to testosterone propionate from the *Sample solution*

$R_S$  = peak response ratio of clotrimazole related compound A to testosterone propionate from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole Related Compound A RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole related compound A in the *Sample solution* (mg/mL)

Acceptance criteria: NMT 5%

#### SPECIFIC TESTS

- **MICROBIAL ENUMERATION TESTS** <61> and **TESTS FOR SPECIFIED MICROORGANISMS** <62>: It meets the requirements for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

- **PH** <791>: 5.0–7.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature between 2° and 30°.

#### • USP REFERENCE STANDARDS (11)

USP Clotrimazole RS

USP Clotrimazole Related Compound A RS  
(o-Chlorophenyl)diphenylmethanol.

C<sub>19</sub>H<sub>15</sub>ClO 294.78

## Clotrimazole Lozenges

#### DEFINITION

Clotrimazole Lozenges contain NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole (C<sub>22</sub>H<sub>17</sub>ClN<sub>2</sub>) in a suitable molded base.

#### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

#### ASSAY

##### • PROCEDURE

**Buffer:** 0.3 g/L of anhydrous monobasic sodium phosphate and 0.35 g/L of anhydrous dibasic sodium phosphate in water. The resulting solution has a pH of 6.6–7.0.

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Diluent:** Acetonitrile and water (1:1)

**Standard solution:** 0.2 mg/mL of USP Clotrimazole RS in *Diluent*

**Sample solution:** Nominally 0.2 mg/mL of clotrimazole in *Diluent* prepared as follows. Transfer a portion of powdered Lozenges (from NLT 20 Lozenges) equivalent to 5 mg of clotrimazole to a 25-mL volumetric flask. Dilute with *Diluent* to volume. Sonicate for about 10 min, and centrifuge at 3500 rpm for about 15 min at ambient temperature to obtain a clear supernatant. Use the clear supernatant for injection.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 206 nm. For *Identification test B* use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 5-μm packing L85

Flow rate: 1 mL/min

Injection volume: 8 μL

Run time: 1.25 times the retention time of clotrimazole

#### System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clotrimazole (C<sub>22</sub>H<sub>17</sub>ClN<sub>2</sub>) in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clotrimazole from the *Sample solution*

$r_S$  = peak response of clotrimazole from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)



Acceptance criteria: 90.0%–110.0%

## PERFORMANCE TESTS

### DISSOLUTION (711)

Medium: 0.1 N hydrochloric acid; 500 mL, deaerated

Apparatus 2: 50 rpm

Time: 45 min

Determine the amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ) dissolved by using the following method.

Buffer A: 4.4 mg/mL of dibasic potassium phosphate in water

Buffer B: 17.4 mg/mL of dibasic potassium phosphate in water

Mobile phase: Methanol and Buffer A (4:1)

Diluent: Methanol and Buffer B (60:40)

Standard stock solution: 0.02 mg/mL of USP Clotrimazole RS in Medium

Standard solution: 4 µg/mL from the Standard stock solution in Diluent

Sample solution: Withdraw 25 mL of the solution under test from the vessel. Pass through a polyvinylidene difluoride filter of 0.45-µm pore size, and discard the first 10 mL of the filtrate. Transfer 5.0 mL of filtrate to a 25-mL volumetric flask, and dilute with Diluent to volume.

### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 215 nm

Column: 3.9-mm × 7.5-cm; packing L1

Flow rate: 1.0 mL/min

Injection volume: 20 µL

### System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times D \times V \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of the Standard solution (mg/mL)

$L$  = label claim of clotrimazole (mg/Lozenge)

$D$  = dilution factor for the Sample solution, 5

$V$  = volume of Medium, 500 mL

Tolerances: NLT 80% (Q) of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### ORGANIC IMPURITIES

Buffer, Mobile phase, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

Standard solution: 1 µg/mL each of USP Clotrimazole RS, USP Clotrimazole Related Compound A RS, and USP Imidazole RS in Diluent

### System suitability

Sample: Standard solution

Suitability requirements

Resolution: NLT 4.0 between clotrimazole related compound A and imidazole peaks; NLT 4.0 between clotrimazole and clotrimazole related compound A peaks

Relative standard deviation: NMT 2.0% for clotrimazole, clotrimazole related compound A, and imidazole

## Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of each specified impurity in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the corresponding specified impurity from the Sample solution

$r_S$  = peak response of the corresponding specified impurity from the Standard solution

$C_S$  = concentration of the corresponding USP Reference Standard in the Standard solution (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the Sample solution (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Lozenges taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity from the Sample solution

$r_S$  = peak response of clotrimazole from the Standard solution

$C_S$  = concentration of USP Clotrimazole RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the Sample solution (mg/mL)

Acceptance criteria: See Table 1. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Imidazole	0.5	0.5
Clotrimazole related compound A	0.7	0.5
Clotrimazole	1.0	—
Any unspecified impurity	—	0.2
Total impurities	—	2.0

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

- **USP REFERENCE STANDARDS (11)**

USP Clotrimazole RS

USP Clotrimazole Related Compound A RS

(o-Chlorophenyl)diphenylmethanol.

$C_{19}H_{15}ClO$  294.78

USP Imidazole RS

## Clotrimazole Topical Solution

### DEFINITION

Clotrimazole Topical Solution is a solution of Clotrimazole in a suitable nonaqueous, hydrophilic solvent. It contains NLT 90.0% and NMT 115.0% of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.
- **B.** The UV spectrum of the clotrimazole peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.



**ASSAY****• PROCEDURE**

**Buffer:** 0.3 g/L of monobasic sodium phosphate, anhydrous and 0.35 g/L of dibasic sodium phosphate, anhydrous in water. The resulting solution has a pH of 6.6–7.0.

**Mobile phase:** Acetonitrile and *Buffer* (50:50)

**Diluent:** Acetonitrile and water (50:50)

**Standard solution:** 0.2 mg/mL of USP Clotrimazole RS in *Diluent*

**Sample solution:** Nominally equivalent to 0.2 mg/mL of clotrimazole from Topical Solution in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 206 nm. For *Identification test B*, use a diode array detector in the range of 200–300 nm.

**Column:** 4.6-mm × 15-cm; 5-μm packing L85

**Flow rate:** 1 mL/min

**Injection volume:** 8 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ) in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clotrimazole from the *Sample solution*

$r_S$  = peak response of clotrimazole from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–115.0%

**IMPURITIES****• ORGANIC IMPURITIES**

**Buffer, Mobile phase, Diluent, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.001 mg/mL each of USP Clotrimazole RS, USP Imidazole RS, and USP Clotrimazole Related Compound A RS in *Diluent*

**System suitability**

**Sample:** *Standard solution*

[NOTE—See *Table 1* for the relative retention times.]

**Suitability requirements**

**Resolution:** NLT 4 between imidazole and clotrimazole related compound A, and between clotrimazole and clotrimazole related compound A

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of clotrimazole related compound A and imidazole in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clotrimazole related compound A or imidazole from the *Sample solution*

$r_S$  = peak response of clotrimazole related compound A or imidazole from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole Related Compound A RS or USP Imidazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Topical Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each unspecified impurity from the *Sample solution*

$r_S$  = peak response of the clotrimazole from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 1*. Disregard any impurity peak less than 0.05%.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Imidazole	0.5	0.5
Clotrimazole related compound A	0.7	0.5
Clotrimazole	1	—
Any unspecified impurity	—	0.2
Total impurities	—	2.0

**ADDITIONAL REQUIREMENTS**

**• PACKAGING AND STORAGE:** Preserve in tight containers at a temperature between 2° and 30°.

**• USP REFERENCE STANDARDS (11)**

USP Clotrimazole RS

USP Clotrimazole Related Compound A RS  
(*o*-Chlorophenyl)diphenylmethanol.

$C_{19}H_{15}ClO$  294.78

USP Imidazole RS

$C_3H_4N_2$  68.08

**Clotrimazole Vaginal Inserts****DEFINITION**

Clotrimazole Vaginal Inserts contain NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ).

**IDENTIFICATION**

**• A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**• B.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Buffer:** 0.3 g/L of anhydrous monobasic sodium phosphate and 0.35 g/L of anhydrous dibasic sodium phosphate in water. The resulting solution has a pH of 6.6–7.0.

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Diluent:** Acetonitrile and water (1:1)

**Standard solution:** 0.2 mg/mL of USP Clotrimazole RS in *Diluent*

**Sample solution:** Nominally 0.2 mg/mL of clotrimazole in *Diluent* prepared as follows. Transfer a portion of



powdered Vaginal Inserts (from NLT 20 Vaginal Inserts) equivalent to 5 mg of clotrimazole to a 25-mL volumetric flask. Dilute with *Diluent* to volume. Sonicate for about 10 min, and centrifuge at 3500 rpm for about 15 min at ambient temperature to obtain a clear supernatant. Use the clear supernatant for injection.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 206 nm. For *Identification* test B use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 5-μm packing L85

Flow rate: 1 mL/min

Injection volume: 8 μL

Run time: 1.25 times the retention time of clotrimazole

#### System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.0%

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ) in the portion of Vaginal Inserts taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clotrimazole from the *Sample solution*

$r_S$  = peak response of clotrimazole from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISINTEGRATION (701)

Time: 20 min

Acceptance criteria: Meet the requirements

##### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

Buffer, Mobile phase, Diluent, Sample solution, and Chromatographic system: Proceed as directed in the *Assay*.

Standard solution: 1 μg/mL each of USP Clotrimazole RS, USP Clotrimazole Related Compound A RS, and USP Imidazole RS in *Diluent*

#### System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 4.0 between clotrimazole related compound A and imidazole peaks; NLT 4.0 between clotrimazole and clotrimazole related compound A peaks

Relative standard deviation: NMT 2.0% for clotrimazole, clotrimazole related compound A, and imidazole

#### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Vaginal Inserts taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the corresponding specified impurity from the *Sample solution*

$r_S$  = peak response of the corresponding specified impurity from the *Standard solution*

$C_S$  = concentration of the corresponding USP Reference Standard in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified impurity in the portion of Vaginal Inserts taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified impurity from the *Sample solution*

$r_S$  = peak response of clotrimazole from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*. Disregard any impurity peak less than 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Imidazole	0.5	0.5
Clotrimazole related compound A	0.7	0.5
Clotrimazole	1.0	—
Any unspecified impurity	—	0.2
Total impurities	—	2.0

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

##### • USP REFERENCE STANDARDS (11)

USP Clotrimazole RS

USP Clotrimazole Related Compound A RS

(*o*-Chlorophenyl)diphenylmethanol.

$C_{19}H_{15}ClO$  294.78

USP Imidazole RS

## Clotrimazole and Betamethasone Dipropionate Cream

#### DEFINITION

Clotrimazole and Betamethasone Dipropionate Cream contains NLT 90.0% and NMT 110.0% of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ) and an amount of betamethasone dipropionate equivalent to NLT 90.0% and NMT 110.0% of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ), in a suitable cream base.

#### IDENTIFICATION

• **A.** The retention times of the major peaks for clotrimazole and betamethasone dipropionate of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay* for clotrimazole and betamethasone.

#### ASSAY

##### • PROCEDURE

Buffer: 6.6 g/L of dibasic ammonium phosphate in water

Mobile phase: Prepare a mixture of methanol and Buffer (7:3), and adjust with phosphoric acid to a pH of  $7.0 \pm 0.2$ . Pass through a membrane filter having a 0.45-μm or finer pore size, and degas.



**Internal standard solution:** 0.15 mg/mL of progesterone in alcohol

**Clotrimazole stock solution:** 5 mg/mL of USP Clotrimazole RS in alcohol

**Betamethasone dipropionate stock solution:** 6.4/ mg/mL of USP Betamethasone Dipropionate RS in alcohol,  $f$  being the ratio of the labeled amount of betamethasone (in mg/g) to the labeled amount of clotrimazole (in mg/g) in the Cream

**Clotrimazole related compound A stock solution:** 0.5 mg/mL of USP Clotrimazole Related Compound A RS in methanol

**Standard solution:** Transfer 1.0 mL of *Clotrimazole related compound A stock solution* to a suitable container, and evaporate to dryness in a water bath at room temperature under a stream of nitrogen. To the residue add 2.0 mL each of *Clotrimazole stock solution*, *Betamethasone dipropionate stock solution*, and *Internal standard solution*.

**Sample solution:** Weigh a portion of Cream equivalent to 10 mg of clotrimazole, and transfer to a screw-capped, 50-mL centrifuge tube. Add 2.0 mL of *Internal standard solution* and 4.0 mL of alcohol, place the cap on the tube, and heat at 60° in a water bath for 10 min, with occasional shaking. Remove the tube from the bath, cool in an ice bath for 20 min, and promptly centrifuge. Transfer a portion of the supernatant to a test tube, and use as the *Sample solution*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 10-μm packing L1

**Flow rate:** 1.7 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for betamethasone dipropionate, clotrimazole related compound A, progesterone, and clotrimazole are about 1.0, 1.2, 1.4, and 1.7, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.0 between betamethasone dipropionate and clotrimazole related compound A, NLT 1.5 between clotrimazole related compound A and progesterone, and NLT 1.8 between progesterone and clotrimazole

**Relative standard deviation:** NMT 2.0% determined from clotrimazole and betamethasone dipropionate and NMT 4.0% determined from clotrimazole related compound A

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of clotrimazole to progesterone from the *Sample solution*

$R_S$  = peak response ratio of clotrimazole to progesterone from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole RS in the *Clotrimazole stock solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

Calculate the percentage of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak response ratio of betamethasone dipropionate to progesterone from the *Sample solution*

$R_S$  = peak response ratio of betamethasone dipropionate to progesterone from the *Standard solution*

$C_S$  = concentration of USP Betamethasone Dipropionate RS in the *Betamethasone dipropionate stock solution* (mg/mL)

$C_U$  = nominal concentration of betamethasone in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of betamethasone, 392.46

$M_{r2}$  = molecular weight of betamethasone dipropionate, 504.60

**Acceptance criteria:** 90.0%–110.0% of the labeled amount of clotrimazole ( $C_{22}H_{17}ClN_2$ ); 90.0%–110.0% of the labeled amount of betamethasone ( $C_{22}H_{29}FO_5$ )

#### IMPURITIES

##### • ORGANIC IMPURITIES: LIMIT OF CLOTRIMAZOLE RELATED COMPOUND A

Buffer, Mobile phase, Internal standard solution, Clotrimazole stock solution, Betamethasone dipropionate stock solution, Clotrimazole related compound A stock solution, Standard solution, Sample solution, Chromatographic system, and System suitability: Use as directed in the Assay.

**Analysis:** Using the chromatograms of the *Standard solution* and *Sample solution* as obtained in the Assay, calculate the percentage of clotrimazole related compound A in the portion of Cream taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of clotrimazole related compound A to progesterone from the *Sample solution*

$R_S$  = peak response ratio of clotrimazole related compound A to progesterone from the *Standard solution*

$C_S$  = concentration of USP Clotrimazole Related Compound A RS in the *Clotrimazole related compound A stock solution* (mg/mL)

$C_U$  = nominal concentration of clotrimazole in the *Sample solution* (mg/mL)

**Acceptance criteria:** NMT 5.0%

#### PERFORMANCE TESTS

- **MINIMUM FILL (755):** Meets the requirements

#### SPECIFIC TESTS

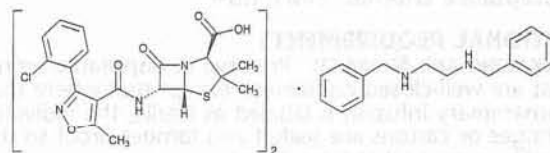
- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in collapsible tubes or tight containers.
- **USP REFERENCE STANDARDS (11)**
  - USP Betamethasone Dipropionate RS
  - USP Clotrimazole RS
  - USP Clotrimazole Related Compound A RS
  - (o-Chlorophenyl)diphenylmethanol.
  - $C_{19}H_{15}ClO$  294.78



## Cloxacillin Benzathine



$(C_{19}H_{18}ClN_3O_5S)_2 \cdot C_{16}H_{20}N_2$  1112.11  
 4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl]carbonyl]amino]-3,3-dimethyl-7-oxo-, [2*S*-(2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ )], compd. with *N,N'*-bis(phenylmethyl)-1,2-ethanediamine (2:1);  
 (2*S*,5*R*,6*R*)-6-[3-(*o*-Chlorophenyl)-5-methyl-4-isoxazole-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid compound with *N,N'*-dibenzylethylenediamine (2:1) [23736-58-5].

### DEFINITION

Cloxacillin Benzathine has a potency equivalent to NLT 704  $\mu$ g and NMT 821  $\mu$ g of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ) per mg, calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. ULTRAVIOLET ABSORPTION** (197U)

**Sample solution:** To 20 mg of Cloxacillin Benzathine add 5 mL of 5 N sodium hydroxide. Heat the solution on a steam bath for 20 min, and cool. Add 1 mL of this solution to 10 mL of 1.2 N sulfuric acid in a separator, and extract with 50 mL of ether. Wash the ether extract with 30 mL of water, and extract the ether layer with 50 mL of 0.1 N sodium hydroxide.

**Standard solution:** Use 15 mg of USP Cloxacillin Sodium RS, and follow the same procedures as described in the *Sample solution*.

Acceptance criteria: Meets the requirements

### ASSAY

#### • PROCEDURE

**Buffer:** 0.1 M monobasic sodium phosphate in water, prepared by dissolving 55.2 g of monobasic sodium phosphate in water, and diluting with water to 4 L  
**Mobile phase:** Acetonitrile and *Buffer* (1:3). Adjust with phosphoric acid or 1 N sodium hydroxide to a pH of  $4.6 \pm 0.2$ . Pass through a 0.45- $\mu$ m nylon filter, and degas. [NOTE—The retention time of cloxacillin is very sensitive to the acetonitrile content of the *Mobile phase*.]  
**Diluent:** 0.05 M monobasic sodium phosphate in water. Mix acetonitrile and the resulting solution (2:3). Adjust with phosphoric acid or 1 N sodium hydroxide to a pH of 6.4.

**Standard solutions:** In duplicate, 112  $\mu$ g/mL of USP Cloxacillin Sodium RS in *Diluent*  
**Sample solutions:** In duplicate, 128  $\mu$ g/mL of Cloxacillin Benzathine in *Diluent*

**Chromatographic system**  
 (See *Chromatography* (621), *System Suitability*).  
**Mode:** LC  
**Detector:** UV 220 nm  
**Column:** 4.6-mm  $\times$  25-cm; 10- $\mu$ m packing L1  
**Column temperature:** 40°  
**Flow rate:** 1.5 mL/min  
**Injection volume:** 10  $\mu$ L

**System suitability**

**Samples:** *Standard solutions*

**Suitability requirements:** Peak areas of the two *Standard solutions* agree within 98%–102%.

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2%

### Analysis

**Samples:** *Standard solutions* and *Sample solutions*

Calculate the quantity, in  $\mu$ g, of cloxacillin

( $C_{19}H_{18}ClN_3O_5S$ ) in each mg of Cloxacillin Benzathine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = average peak areas of cloxacillin from the *Sample solutions*

$r_S$  = average peak areas of cloxacillin from the *Standard solutions*

$C_S$  = concentration of USP Cloxacillin Sodium RS in the *Standard solutions* ( $\mu$ g/mL)

$C_U$  = concentration of Cloxacillin Benzathine in the *Sample solutions* ( $\mu$ g/mL)

$P$  = assigned potency of USP Cloxacillin Sodium RS ( $\mu$ g of cloxacillin per mg)

Acceptance criteria: 704–821  $\mu$ g/mg on the anhydrous basis

### SPECIFIC TESTS

- **CRYSTALLINITY** (695): Meets the requirements

- **pH** (791)

**Sample solution:** 10 mg/mL of suspension

Acceptance criteria: 3.0–6.5

- **STERILITY TESTS** (71): Where the label states that Cloxacillin Benzathine is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined*, *Direct Inoculation of the Culture Medium*, except use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, and shake the tubes once daily.

- **WATER DETERMINATION, Method I** (921): NMT 5.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **LABELING:** Label it to indicate that it is for veterinary use only. Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.
- **USP REFERENCE STANDARDS** (11)  
 USP Cloxacillin Benzathine RS  
 USP Cloxacillin Sodium RS

## Cloxacillin Benzathine Intramammary Infusion

### DEFINITION

Cloxacillin Benzathine Intramammary Infusion is a suspension of Cloxacillin Benzathine in a suitable oil vehicle. It has a potency equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ).

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)

**Sample:** Transfer a quantity of Intramammary Infusion, equivalent to 500 mg of cloxacillin, to a 50-mL centrifuge tube. Add 25 mL of toluene, mix, and centrifuge. Decant and discard the toluene. Wash the residue with four 25-mL portions of toluene, sonicating for 30 s after each addition of toluene. Dry the residue under vacuum over silica gel.



Acceptance criteria: Meets the requirements

## ASSAY

### PROCEDURE

**Buffer:** 0.1 M monobasic sodium phosphate in water prepared by dissolving 55.2 g of monobasic sodium phosphate in water, and diluting with water to 4 L  
**Mobile phase:** Acetonitrile and *Buffer* (1:3). Adjust with phosphoric acid or 1 N sodium hydroxide to a pH of  $4.6 \pm 0.2$ . Pass through a 0.45- $\mu$ m nylon filter, and degas. [NOTE—The retention time of cloxacillin is very sensitive to the acetonitrile content of the *Mobile phase*.]  
**Diluent:** 0.05 M monobasic sodium phosphate in water. Mix acetonitrile and the resulting solution (2:3). Adjust with phosphoric acid or 1 N sodium hydroxide to a pH of 6.4.

**Standard solutions:** In duplicate, 112  $\mu$ g/mL of USP Cloxacillin Sodium RS in *Diluent*

**Sample solutions:** Nominally 100  $\mu$ g/mL of cloxacillin prepared as follows. In duplicate, quantitatively express the entire contents of a syringe of Intramammary Infusion into a 500-mL volumetric flask. Add 300 mL of methanol, and stir for  $45 \pm 1$  min. Dilute with methanol to volume, and stir for an additional  $10 \pm 1$  min. Immediately transfer 45 mL of the resulting solution to a 50-mL polypropylene centrifuge tube, and centrifuge for 10 min. From the supernatant remove an aliquot, and dilute with a sufficient volume of *Diluent* to prepare a solution containing nominally 100  $\mu$ g/mL of cloxacillin.

### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm  $\times$  25-cm; 10- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 10  $\mu$ L

### System suitability

**Samples:** *Standard solutions*

**Suitability requirements:** Peak areas of the two *Standard solutions* agree within 98%–102%.

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2%

### Analysis

**Samples:** *Standard solutions* and *Sample solutions*

Calculate the percentage of the labeled amount of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ) in each syringe of Intramammary Infusion taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = average peak areas of cloxacillin from the *Sample solutions*

$r_S$  = average peak areas of cloxacillin from the *Standard solutions*

$C_S$  = concentration of cloxacillin in the *Standard solutions* ( $\mu$ g/mL)

$C_U$  = nominal concentration of cloxacillin in the *Sample solutions* ( $\mu$ g/mL)

Acceptance criteria: 90.0%–120.0%

## SPECIFIC TESTS

- STERILITY TESTS** <71>: Where the label states that it is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*, except use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, and shake the tubes once daily.

### WATER DETERMINATION, Method I <921>

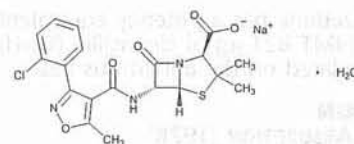
**Analysis:** Use 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.

Acceptance criteria: NMT 1.0%

## ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in disposable syringes that are well-closed containers, except that where the Intramammary Infusion is labeled as sterile, the individual syringes or cartons are sealed and tamper-proof so that sterility is assured at time of use.
- LABELING:** Label it to indicate that it is for veterinary use only. Intramammary Infusion that is sterile may be so labeled.
- USP REFERENCE STANDARDS** <11>  
 USP Cloxacillin Benzathine RS  
 USP Cloxacillin Sodium RS

## Cloxacillin Sodium



$C_{19}H_{17}ClN_3NaO_5S \cdot H_2O$  475.88

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[[3-(2-chlorophenyl)-5-methyl-4-isoxazolyl]carbonyl]amino]-3,3-dimethyl-7-oxo-, monosodium salt, monohydrate, [2S-(2 $\alpha$ ,5 $\alpha$ ,6 $\beta$ )]-;  
 Monosodium (2S,5R,6R)-6-[[[3-(o-chlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate [7081-44-9].

Anhydrous

$C_{19}H_{17}ClN_3NaO_5S$  457.87  
 [642-78-4].

## DEFINITION

Cloxacillin Sodium contains the equivalent of NLT 825  $\mu$ g/mg of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ).

## IDENTIFICATION

- A. INFRARED ABSORPTION** <197K>
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- C. IDENTIFICATION TESTS—GENERAL, Sodium** <191>: Meets the requirements

## ASSAY

### PROCEDURE

Protect solutions containing cloxacillin from light.

**Solution A:** 1.18 g/L of sodium 1-hexanesulfonate monohydrate and 0.8 mL/L of ammonium hydroxide in water, adjusted with phosphoric acid to a pH of 2.9–3.1

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	45	55
2	45	55
2.5	35	65
5	35	65



Return to the original conditions and re-equilibrate the system.

**Diluent:** Acetonitrile and water (50:50)

**System suitability stock solution:** 0.1 mg/mL of USP Cloxacillin Related Compound D RS in *Diluent*. Sonicate as needed to dissolve.

**System suitability solution:** 0.001 mg/mL of USP Cloxacillin Related Compound D RS from *System suitability stock solution* and 0.1 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Store this solution at 4°.

**Standard solution:** 0.1 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

**Sample solution:** 0.1 mg/mL of Cloxacillin Sodium in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 225 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Temperatures**

**Column:** 40°

**Autosampler:** 4°

**Flow rate:** 1.5 mL/min

**Injection volume:** 10 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cloxacillin and cloxacillin related compound D are about 1.0 and 1.1, respectively.]

#### Suitability requirements

**Resolution:** NLT 1.5 between cloxacillin and cloxacillin related compound D, *System suitability solution*

**Tailing factor:** 0.8–1.5, *Standard solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in μg/mg, of cloxacillin (C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S) in the portion of Cloxacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cloxacillin Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cloxacillin Sodium in the *Sample solution* (mg/mL)

$P$  = potency of cloxacillin in USP Cloxacillin Sodium RS (μg/mg)

**Acceptance criteria:** NLT 825 μg/mg

#### IMPURITIES

##### • ORGANIC IMPURITIES

Protect solutions containing cloxacillin from light.

**Solution A, Solution B, Diluent, and Chromatographic system:** Proceed as directed in the Assay.

**Mobile phase:** See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	80	20
30	35	65

Return to the original conditions and re-equilibrate the system.

**System suitability stock solution:** 0.1 mg/mL of USP Cloxacillin Related Compound D RS in *Diluent*

**System suitability solution:** 0.01 mg/mL of USP Cloxacillin Related Compound D RS from *System suitability stock solution* and 1 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Store this solution at 4°.

**Standard solution:** 0.01 mg/mL of USP Cloxacillin Sodium RS in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

**Sample solution:** 1 mg/mL of Cloxacillin Sodium in *Diluent*. Sonicate as needed to dissolve. Store this solution at 4°.

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between cloxacillin related compound D and cloxacillin, *System suitability solution*

**Tailing factor:** 0.8–1.5, *Standard solution*

**Relative standard deviation:** NMT 2.5%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cloxacillin Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times (F_1/F_2) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cloxacillin Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cloxacillin Sodium in the *Sample solution* (mg/mL)

$P$  = potency of cloxacillin in USP Cloxacillin Sodium RS (μg/mg)

$F_1$  = conversion factor, 0.001 mg/μg

$F_2$  = relative response factor (see Table 3)

**Acceptance criteria:** See Table 3. The reporting threshold is 0.05%.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Amoxicillin related compound A <sup>a</sup>	0.12	0.24	1.0
Cloxacillin penicilloic acid <sup>b</sup>	0.49	0.65	1.0
Cloxacillin penilloic acid <sup>c,d</sup>	0.70 0.72	1.0	1.0
Cloxacillin related compound D <sup>e</sup>	0.89	1.0	1.0
Cloxacillin	1.0	—	—
Ticlocxacillin <sup>f</sup>	1.18	1.0	1.0
Cloxacillin penicillamides	1.25	1.0	1.0

<sup>a</sup> 6-Aminopenicillanic acid; (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>b</sup> (4S)-2-[(Carboxy[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido]methyl)-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>c</sup> (4S)-2-[(3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>d</sup> The system resolves two isomers. The limit is for the sum of the isomers.

<sup>e</sup> 3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxylic acid.

<sup>f</sup> (2R,5R,6R)-6-[3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>g</sup> (2S,5R,6R)-6-[(2S,5R,6R)-6-[3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>h</sup> (2S,5R,6R)-6-[(R)-2-[(2R,4S)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido]acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.



Table 3 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Cloxacillin penicilloic penicillamide <sup>a</sup>	1.54	1.0	1.0
Any individual unspecified impurity	—	1.0	1.0
Total impurities	—	—	5.0

<sup>a</sup> 6-Aminopenicillanic acid; (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>b</sup> (4S)-2-[(Carboxy[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido]methyl)-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>c</sup> (4S)-2-[(3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido)methyl]-5,5-dimethylthiazolidine-4-carboxylic acid.

<sup>d</sup> The system resolves two isomers. The limit is for the sum of the isomers.

<sup>e</sup> 3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxylic acid.

<sup>f</sup> (2R,5R,6R)-6-[3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>g</sup> (2S,5R,6R)-6-[(2S,5R,6R)-6-[3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

<sup>h</sup> (2S,5R,6R)-6-[(R)-2-[(2R,4S)-4-Carboxy-5,5-dimethylthiazolidin-2-yl]-2-[3-(2-chlorophenyl)-5-methylisoxazole-4-carboxamido]acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

- **DIMETHYLANILINE (223):** Meets the requirements

#### SPECIFIC TESTS

- **CRYSTALLINITY (695):** Meets the requirements

- **PH (791)**

Sample solution: 10 mg/mL in water

Acceptance criteria: 4.5–7.5

- **STERILITY TESTS (71):** Meets the requirements where the label states that Cloxacillin Sodium is sterile. If the test for *Direct Inoculation of the Culture Medium* is used, perform the procedure as directed in the chapter with the following exceptions. Use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube. Use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube. Shake the tubes once daily.
- **WATER DETERMINATION, Method I (921):** 3.0%–5.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at a temperature not exceeding 25°.
- **LABELING:** Where it is intended for use in preparing sterile dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of sterile dosage forms.
- **USP REFERENCE STANDARDS (11)**
  - USP Cloxacillin Related Compound D RS
  - 3-(2-Chlorophenyl)-5-methylisoxazole-4-carboxylic acid.
  - C<sub>11</sub>H<sub>8</sub>ClNO<sub>3</sub> 237.64
  - USP Cloxacillin Sodium RS

#### ASSAY

##### • PROCEDURE

**Buffer:** 0.02 M monobasic potassium phosphate in water, adjusted with 2 N sodium hydroxide to a pH of 6.8

**Mobile phase:** Acetonitrile and Buffer (20:80)

**Standard solution:** 0.55 mg/mL of USP Cloxacillin Sodium RS in Buffer

**Sample solution:** Nominally 0.5 mg/mL of cloxacillin in Buffer, prepared as follows. Mix the contents of NLT 10 Capsules. Transfer a suitable portion of the powder to a volumetric flask, dilute with Buffer to volume, and stir for 10 min. Pass a portion of the solution through a suitable filter, discarding the first 5 mL of the filtrate. Use the clear filtrate.

##### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 225 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

##### System suitability

**Sample:** Standard solution

**Suitability requirements**

**Tailing factor:** NMT 1.8

**Relative standard deviation:** NMT 2.0%

##### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of cloxacillin (C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S) in the portion of Capsules taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times P \times F \times 100$$

$r_u$  = peak response from the Sample solution

$r_s$  = peak response from the Standard solution

$C_s$  = concentration of USP Cloxacillin Sodium RS in the Standard solution (mg/mL)

$C_u$  = nominal concentration of cloxacillin in the Sample solution (mg/mL)

$P$  = potency of cloxacillin in USP Cloxacillin Sodium RS (µg/mg)

$F$  = conversion factor, 0.001 mg/µg

**Acceptance criteria:** 90.0%–120.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** 0.05 M pH 6.8 potassium phosphate buffer; 900 mL

**Apparatus 1:** 100 rpm

**Time:** 30 min

**Buffer, Mobile phase, Chromatographic system, and**

**System suitability:** Proceed as directed in the Assay.

**Standard solution:** USP Cloxacillin Sodium RS in Medium

**Sample solution:** Sample per the chapter.

**Tolerances:** NLT 80% (Q) of the labeled amount of cloxacillin (C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## Cloxacillin Sodium Capsules

#### DEFINITION

Cloxacillin Sodium Capsules contain the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cloxacillin (C<sub>19</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>5</sub>S).



**SPECIFIC TESTS**

- **WATER DETERMINATION** (921), *Method I*: NMT 5.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Cloxacillin Sodium RS

## Cloxacillin Sodium Intramammary Infusion

**DEFINITION**

Cloxacillin Sodium Intramammary Infusion is a suspension of Cloxacillin Sodium in a suitable natural or chemically modified vegetable oil vehicle with a suitable dispersing agent. It has a potency equivalent to NLT 90.0% and NMT 120.0% of the labeled amount of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ).

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)

**Sample**: Transfer a quantity of Intramammary Infusion, equivalent to 500 mg of cloxacillin, to a 50-mL centrifuge tube. Add 15 mL of isooctane, mix, and centrifuge. Decant and discard the isooctane. Wash the residue with two 15-mL portions of isooctane and two 15-mL portions of ethyl ether, and discard the washings. Dry the residue in a current of air.

**Acceptance criteria**: Meets the requirements

**ASSAY**

- **PROCEDURE**

(See *Antibiotics—Microbial Assays* (81).)

**Sample solution**: Expel the contents of 1 syringe of Intramammary Infusion into a high-speed glass blender jar containing 499.0 mL of *Buffer B.1* and 1.0 mL of polysorbate 80, and blend for 3–5 min. Allow to stand for 10 min, and dilute a measured volume of the aqueous phase with *Buffer B.1* to obtain a test dilution having a concentration assumed to be equal to the median dose level of the Standard.

**Analysis**: Proceed as directed in the chapter for Cloxacillin.

**Acceptance criteria**: 90.0%–120.0%

**SPECIFIC TESTS**

- **STERILITY TESTS** (71): Where the label states that it is sterile, it meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*, except use Fluid Thioglycollate Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, use Soybean–Casein Digest Medium containing polysorbate 80 solution (1 in 200) and an amount of sterile penicillinase sufficient to inactivate the cloxacillin in each tube, and shake the tubes once daily.
- **WATER DETERMINATION, Method I** (921)  
**Analysis**: Use 20 mL of a mixture of toluene and methanol (7:3) in place of methanol in the titration vessel.  
**Acceptance criteria**: NMT 1.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in disposable syringes that are well-closed containers, except that where the In-

tramammary Infusion is labeled as sterile, the individual syringes or cartons are sealed and tamper-proof so that sterility is assured at time of use.

- **LABELING**: Label it to indicate that it is for veterinary use only. Intramammary Infusion that is sterile may be so labeled.
- **USP REFERENCE STANDARDS** (11)  
USP Cloxacillin Sodium RS

## Cloxacillin Sodium for Oral Solution

**DEFINITION**

Cloxacillin Sodium for Oral Solution is a dry mixture of Cloxacillin Sodium and one or more suitable buffers, colors, flavors, and preservatives. It contains the equivalent of NLT 90.0% and NMT 120.0% of the labeled amount of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ).

**ASSAY**

- **PROCEDURE**

**Buffer**: 0.02 M of monobasic potassium phosphate in water, adjusted with 2 N sodium hydroxide to a pH of 6.8

**Mobile phase**: Acetonitrile and *Buffer* (20:80)

**Standard solution**: 0.55 mg/mL of USP Cloxacillin Sodium RS in *Buffer*

**Sample solution**: Nominally 0.5 mg/mL of cloxacillin in *Buffer*, prepared as follows. Constitute Cloxacillin Sodium for Oral Solution as directed in the labeling.

Transfer a suitable portion of the resulting solution to a volumetric flask, dilute with *Buffer* to volume, mix, and stir for 15 min. Pass a portion of the solution through a suitable filter, discarding the first 5 mL of the filtrate. Use the clear filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode**: LC

**Detector**: UV 225 nm

**Column**: 4.6-mm × 25-cm; packing L1

**Flow rate**: 1 mL/min

**Injection volume**: 20 µL

**System suitability**

**Sample**: *Standard solution*

**Suitability requirements**

**Tailing factor**: NMT 1.8

**Relative standard deviation**: NMT 2.0%

**Analysis**

**Samples**: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cloxacillin ( $C_{19}H_{18}ClN_3O_5S$ ) in the portion of Cloxacillin Sodium for Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F \times 100$$

- $r_U$  = peak response from the *Sample solution*
- $r_S$  = peak response from the *Standard solution*
- $C_S$  = concentration of USP Cloxacillin Sodium RS in the *Standard solution* (mg/mL)
- $C_U$  = nominal concentration of cloxacillin in the *Sample solution* (mg/mL)
- $P$  = potency of cloxacillin in USP Cloxacillin Sodium RS (µg/mg)
- $F$  = conversion factor, 0.001 mg/µg



Acceptance criteria: 90.0%–120.0%

### PERFORMANCE TESTS

- **DELIVERABLE VOLUME** (698): Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS** (905)

For solids packaged in single-unit containers

Acceptance criteria: Meets the requirements

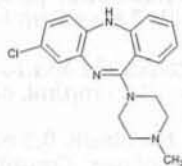
### SPECIFIC TESTS

- **pH** (791)  
Sample solution: Constitute as directed in the labeling.  
Acceptance criteria: 5.0–7.5
- **WATER DETERMINATION** (921), Method I: NMT 1.0%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Cloxacillin Sodium RS

## Clozapine



$C_{18}H_{19}ClN_4$  326.82  
5H-Dibenzo[b,e][1,4]diazepine, 8-chloro-11-(4-methyl-1-piperazinyl)-;  
8-Chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepine [5786-21-0].

### DEFINITION

Clozapine contains NLT 98.0% and NMT 102.0% of clozapine ( $C_{18}H_{19}ClN_4$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

Mobile phase: Methanol, triethylamine, and water (800: 0.75: 200)

Diluent: Methanol and water (80:20)

Standard solution: 0.1 mg/mL of USP Clozapine RS in Diluent

System suitability stock solution: Transfer 10 mg of USP Clozapine RS to a suitable container, add 5 mL of 0.1 N hydrochloric acid, and heat for 2 h at 90°. Transfer this solution to a 100-mL volumetric flask, add 15 mL of water, and dilute with methanol to volume.

System suitability solution: *Standard solution* and *System suitability stock solution* (1:1)

Sample solution: 0.1 mg/mL of Clozapine in Diluent

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 257 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Flow rate: 1 mL/min

Injection size: 10 μL

Run time: 3 times the retention time of clozapine

System suitability

Samples: *Standard solution* and *System Suitability solution*

### Suitability requirements

Resolution: NLT 1.5 between clozapine and any other peak, *System suitability solution*

Tailing factor: NMT 1.5, *Standard solution*

Relative standard deviation: NMT 1.0%, *Standard solution*

### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of clozapine ( $C_{18}H_{19}ClN_4$ ) in the portion of Clozapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of clozapine from the *Sample solution*

$r_S$  = peak response of clozapine from the *Standard solution*

$C_S$  = concentration of USP Clozapine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Clozapine in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

### Delete the following:

- **HEAVY METALS, Method II** (231): 20 ppm (Official 1-Jan-2018)

### ORGANIC IMPURITIES

Diluent: Methanol and water (80:20)

Buffer: 2.0 g/L of monobasic potassium phosphate. Adjust with phosphoric acid (85%) to a pH of 2.4.

[NOTE—The pH of this solution must not be below 2.4.]

Solution A: Filtered and degassed mixture of acetonitrile, methanol, and Buffer (1:1:8)

Solution B: Filtered and degassed mixture of acetonitrile, methanol, and Buffer (4:4:2)

Mobile phase: See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
4	100	0
24	0	100
29	0	100
40	100	0

System suitability solution: Dissolve 4 mg of USP Clozapine Resolution Mixture RS in 4 mL of methanol, add 1 mL of water, and dilute with Diluent to 10 mL.

Standard solution: 0.75 μg/mL of USP Clozapine RS in Diluent

Sample solution: 0.75 mg/mL prepared as follows.

Transfer a suitable quantity of Clozapine to a suitable volumetric flask. Dissolve in 80% of the flask volume of methanol, and dilute with water to volume.

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 257 nm

Column: 4.6-mm × 15-cm; 5-μm packing L1

Flow rate: 1.2 mL/min

Injection size: 20 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times are provided in Table 2.]

Suitability requirements

Resolution: NLT 2.5 between Impurity C and clozapine, *System suitability solution*



Relative standard deviation: NMT 5.0% for clozapine, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

[NOTE—Disregard any peak with an area less than 0.5 times the area of the clozapine peak from the *Standard solution*.]

Calculate the percentage of each related compound and any unknown impurity in the portion of Clozapine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of any impurity from the *Sample solution*

$r_S$  = peak response of clozapine from the *Standard solution*

$C_S$  = concentration of USP Clozapine RS from the *Standard solution* (mg/mL)

$C_U$  = concentration of Clozapine from the *Sample solution* (mg/mL)

$F$  = relative response factor of the impurity (see Table 2)

Acceptance criteria: See Table 2.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Impurity C <sup>a</sup>	0.9	1.0	0.3
Clozapine	1.0	—	—
Impurity D <sup>b</sup>	1.1	0.35	0.2
Impurity A <sup>c</sup>	1.6	1.2	0.1
Impurity B <sup>d</sup>	1.7	1.0	0.2
Individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.6

<sup>a</sup> 8-Chloro-11-(piperazin-1-yl)-5H-dibenzo[b,e][1,4]diazepine.

<sup>b</sup> 4-Chloro-N<sup>1</sup>-(2-[(4-methylpiperazin-1-yl)carbonyl]phenyl)benzene-1,2-diamine.

<sup>c</sup> 8-Chloro-5,10-dihydro-11H-dibenzo[b,e][1,4]diazepin-11-one.

<sup>d</sup> 11,11'-(Piperazine-1,4-diyl)bis(8-chloro-5H-dibenzo[b,e][1,4]diazepine).

#### SPECIFIC TESTS

- LOSS ON DRYING (731):** Dry a sample at 105° for 4 h: it loses NMT 0.5% of its weight.

#### ADDITIONAL REQUIREMENTS

- PACKAGING AND STORAGE:** Preserve in well-closed containers.
- USP REFERENCE STANDARDS (11)**
  - USP Clozapine RS
  - USP Clozapine Resolution Mixture RS
  - Contains the following components: Clozapine.
  - Impurity A: 8-chloro-5,10-dihydro-11H-dibenzo[b,e][1,4]diazepin-11-one.
  - Impurity B: 11,11'-(piperazine-1,4-diyl)bis(8-chloro-5H-dibenzo[b,e][1,4]diazepine).
  - Impurity C: 8-chloro-11-(piperazin-1-yl)-5H-dibenzo[b,e][1,4]diazepine.
  - Impurity D: 4-chloro-N<sup>1</sup>-(2-[(4-methylpiperazin-1-yl)carbonyl]phenyl)benzene-1,2-diamine.

## Clozapine Tablets

#### DEFINITION

Clozapine Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of clozapine (C<sub>18</sub>H<sub>19</sub>ClN<sub>4</sub>).

#### IDENTIFICATION

- A.** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to those of the principal spots of the *Standard solutions*, as obtained in the test for *Organic Impurities*.
- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Mobile phase:** Methanol, triethylamine, and water (800:0.75:200)

**Standard solution:** 0.125 mg/mL of USP Clozapine RS prepared as follows. Transfer the required amount of USP Clozapine RS to a suitable volumetric flask. Dissolve in 80% of the flask volume of methanol. [NOTE—Dissolve the Reference Standard in methanol, and dilute with water to obtain the final concentration. The final solvent composition of methanol and water is about 8:2.]

**System suitability stock solution:** Transfer 10 mg of clozapine to a suitable container, add 5 mL of 0.1 N hydrochloric acid, and heat for 2 h at 90°. Transfer this solution to a 100-mL volumetric flask, add 15 mL of water, and dilute with methanol to volume.

**System suitability solution:** *Standard solution* and *System suitability stock solution* (1:1)

**Sample solution:** Transfer 125 mg of clozapine from a quantity of finely powdered Tablets (NLT 20) to a 1-L volumetric flask. Dissolve in 640 mL of methanol, sonicate for 10 min, dilute with water to volume, mix, and filter.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 257 nm

**Column:** 4.0-mm × 25-cm; packing L7

**Flow rate:** 1 mL/min

**Injection size:** 10 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 1.5 between the clozapine peak and any other peak, *System suitability solution*

**Column efficiency:** NLT 1500 theoretical plates, *Standard solution*

**Relative standard deviation:** NMT 2.0% for replicate injections, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of clozapine (C<sub>18</sub>H<sub>19</sub>ClN<sub>4</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Clozapine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of clozapine in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

#### PERFORMANCE TESTS

##### DISSOLUTION (711)

**Medium:** pH 4.0 acetate buffer prepared as follows.

Dissolve 2 g of sodium hydroxide in 450 mL of water.

Adjust with glacial acetic acid to a pH of 4.0. Dilute to 1 L; 900 mL



Apparatus 1: 100 rpm

Time: 45 min

**Standard solution:** USP Clozapine RS in *Medium* in a concentration similar to the one expected in the *Sample solution*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

**Instrumental conditions**

Mode: UV

Analytical wavelength: 290 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of clozapine ( $C_{18}H_{19}ClN_4$ ) dissolved.

**Tolerances:** NLT 85% (Q) of the labeled amount of clozapine ( $C_{18}H_{19}ClN_4$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Diluent:** Chloroform and methanol (4:1)

**Standard stock solution:** 5.0 mg/mL of USP Clozapine RS in *Diluent*

**Standard solutions:** Dilute portions of the *Standard stock solution* with *Diluent* to obtain the following solutions.

Standard solution	Dilution	Concentration (µg/mL of RS)	Percentage (for comparison with Sample)
A	1 in 200	25	0.5
B	1 in 250	20	0.4
C	1 in 333	15	0.3
D	1 in 500	10	0.2
E	1 in 1000	5	0.1

**Sample solution:** Transfer an equivalent to 125 mg of clozapine from a portion of finely powdered Tablets (NLT 20) to a 25-mL volumetric flask. Dissolve in 20 mL of *Diluent*, shake by mechanical means for 15 min, dilute with *Diluent* to volume, and filter.

**Chromatographic system**  
(See *Chromatography (621)*, *Thin-Layer Chromatography*.)

Mode: TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 20 µL

**Developing solvent system:** *n*-Heptane, chloroform, dehydrated alcohol, and ammonium hydroxide (30:30:30:1)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Examine the plate under short-wavelength UV light, and compare the intensities of any secondary spots of the *Sample solution* with those of the principal spots of the *Standard solutions*.

**Acceptance criteria:** No secondary spot of the *Sample solution* is larger or more intense than the principal spot of *Standard solution A* (NMT 0.5%), and the sum of the intensities of the secondary spots from the *Sample solution* corresponds to NMT 2.0%.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

#### • USP REFERENCE STANDARDS (11)

USP Clozapine RS

### Coal Tar

#### DEFINITION

Coal Tar is the tar obtained as a by-product during the destructive distillation of bituminous coal at temperatures in the range of 900°–1100°. It may be processed further either by extraction with alcohol and suitable dispersing agents and maceration times or by fractional distillation with or without the use of suitable organic solvents.

#### IMPURITIES

##### • RESIDUE ON IGNITION (281)

Sample: 100 mg

Acceptance criteria: NMT 2.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.

### Coal Tar Ointment

» Prepare Coal Tar Ointment as follows.

Coal Tar .....	10 g
Polysorbate 80 .....	5 g
Zinc Oxide Paste .....	985 g
to make .....	1000 g

Blend the Coal Tar with the Polysorbate 80, and incorporate the mixture with the Zinc Oxide Paste.

**Packaging and storage**—Preserve in tight containers.

### Coal Tar Topical Solution

» Prepare Coal Tar Topical Solution as follows.

Coal Tar .....	200 g
Polysorbate 80 .....	50 g
Alcohol, a sufficient quantity, to make .....	1000 mL

Mix the Coal Tar with 500 g of washed sand (see under *Reagents* in the section *Reagents, Indicators, and Solutions*), and add the Polysorbate 80 and 700 mL of Alcohol. Macerate the mixture for 7 days in a closed vessel with frequent agitation. Filter, and rinse the vessel and the filter with sufficient Alcohol to make the product measure 1000 mL.

**Packaging and storage**—Preserve in tight containers.

**Alcohol Determination (611):** between 81.0% and 86.0% of  $C_2H_5OH$ .



## Cyanocobalamin Co 57 Capsules

Vitamin B<sub>12</sub>-<sup>57</sup>Co.

Vitamin B<sub>12</sub>-<sup>57</sup>Co [41559-38-0; 13115-03-2].

» Cyanocobalamin Co 57 Capsules contain Cyanocobalamin in which a portion of the molecules contain radioactive cobalt (<sup>57</sup>Co) in the molecular structure. Each Capsule contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of <sup>57</sup>Co as cyanocobalamin expressed in megabecquerels (microcuries) at the time indicated in the labeling. The cyanocobalamin content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount.

**Specific activity:** not less than 0.02 MBq (0.5 μCi) per μg of cyanocobalamin.

**Packaging and storage**—Preserve in well-closed, light-resistant containers, and store in a cold place.

**Labeling**—Label the Capsules to include the following: the date of calibration; the amount of cyanocobalamin expressed in μg per Capsule; the amount of <sup>57</sup>Co as cyanocobalamin expressed in megabecquerels (microcuries) per Capsule at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of <sup>57</sup>Co is 270.9 days.

**USP Reference standards** (11)—

USP Cyanocobalamin RS

**Radionuclide identification**—A solution of 1 or more Capsules in water responds to the test for *Radionuclide identification* under Cyanocobalamin Co 57 Oral Solution.

**Disintegration** (701): 30 minutes, testing 1 Capsule in 1 N hydrochloric acid maintained at 37 ± 2° as the immersion fluid.

**Uniformity of dosage units:** meet the requirements.

**Procedure for content uniformity**—Determine the instrument response of each of 10 Capsules by measurement in a suitable counting assembly and under identical geometric conditions. Calculate the average radioactivity per Capsule. The radioactivity of none of the Capsules differs by more than 10% from the average. The relative standard deviation is less than 3.5%.

**Radiochemical purity**—Dissolve the contents of 1 Capsule in 1 mL of water, allow to stand for about 10 minutes, and centrifuge. Use the supernatant as the *Test solution*. It meets the requirements of the test for *Radiochemical purity* under Cyanocobalamin Co 57 Oral Solution.

**Radionuclidic purity**—Dissolve the contents of 1 Capsule in 1 mL of water, allow to stand for about 10 minutes, and centrifuge. Use the supernatant. It meets the requirements of the test for *Radionuclidic purity* under Cyanocobalamin Co 57 Oral Solution.

**Content of cyanocobalamin**—Determine the content, in μg per Capsule, of cyanocobalamin as directed under *Vitamin B<sub>12</sub> Activity Assay* (171).

### Change to read:

**Assay for radioactivity**—Using a suitable counting assembly • (CN 1-May-2017), determine the radioactivity, in MBq (μCi) per Capsule, of Cyanocobalamin Co 57 Capsules by use of a calibrated system as directed under *Radioactivity* (821).

## Cyanocobalamin Co 57 Oral Solution

Vitamin B<sub>12</sub>-<sup>57</sup>Co.

Vitamin B<sub>12</sub>-<sup>57</sup>Co [41559-38-0; 13115-03-2].

» Cyanocobalamin Co 57 Oral Solution is a solution suitable for oral administration, containing Cyanocobalamin in which a portion of the molecules contain radioactive cobalt (<sup>57</sup>Co) in the molecular structure. Cyanocobalamin Co 57 Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of <sup>57</sup>Co as cyanocobalamin expressed in megabecquerels (microcuries) per mL at the time indicated in the labeling. The cyanocobalamin content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount. Cyanocobalamin Co 57 Oral Solution contains a suitable antimicrobial agent.

**Specific activity:** not less than 0.02 MBq (0.5 μCi) per μg of cyanocobalamin.

**Packaging and storage**—Preserve in tight containers, protected from light, and store in a cold place.

**Labeling**—Label it to include the following: the date of calibration; the amount of <sup>57</sup>Co as cyanocobalamin expressed as total megabecquerels (microcuries) and as megabecquerels (microcuries) per mL at the time of calibration; the amount of cyanocobalamin expressed in μg per mL; the name and quantity of the added preservative; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of <sup>57</sup>Co is 270.9 days, and directs that the Oral Solution be protected from light.

**USP Reference standards** (11)—

USP Cyanocobalamin RS

**Radionuclide identification** (see *Radioactivity* (821))—Its gamma-ray spectrum is identical to that of a specimen of <sup>57</sup>Co of known purity that exhibits a major photopeak having an energy of 0.122 MeV.

**Uniformity of dosage units** (905)—

FOR ORAL SOLUTION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698)—

FOR ORAL SOLUTION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 4.0 and 5.5.

**Radiochemical purity**—

**Mobile phase**—Prepare a solution of 10.0 g of dibasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 3.5. Prepare a mixture of this solution and methanol (73.5:26.5), mix, and degas. Use within 2 days.

**Test solution**—Use the Oral Solution.

**Standard solution**—Transfer about 10 mg of cyanocobalamin, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 361-nm detector, a gamma detector adjusted for <sup>57</sup>Co and a 4.6-mm × 25-cm stainless steel column that contains 5-μm packing L7. The flow rate is about 1 mL per minute.

**Procedure**—Inject about 100 μL of the *Standard solution* into the chromatograph, record the chromatogram for 30 minutes, and note the retention time of the cyanocobala-



min peak. Inject 100  $\mu\text{L}$  of the *Test solution* into the chromatograph, and record the chromatogram for three times the retention time of cyanocobalamin. Measure the peak areas using the gamma detector, and calculate the percentage of cyanocobalamin present as cyanocobalamin  $^{57}\text{Co}$  in the portion of Oral Solution taken by the formula:

$$100(r_u / r_t)$$

in which  $r_u$  is the peak response for cyanocobalamin  $^{57}\text{Co}$  obtained from the *Test solution*; and  $r_t$  is the total of all the peak area responses in the radiochromatogram obtained from the *Test solution*. Not less than 90% of the total radioactivity is found as cyanocobalamin  $^{57}\text{Co}$ .

**Radionuclidic purity**—Using a suitable calibrated instrument (see *Radioactivity* (821)) and standardized solutions of  $^{58}\text{Co}$ ,  $^{57}\text{Co}$ , and  $^{60}\text{Co}$ , record the gamma spectrum of the Oral Solution. The spectrum does not differ significantly from that of the standardized  $^{57}\text{Co}$  solution. Determine the relative amounts of  $^{58}\text{Co}$ ,  $^{57}\text{Co}$ , and  $^{60}\text{Co}$  present. Cobalt 58 has a half-life of 70.9 days, and its presence is shown by 0.511-MeV and 0.811-MeV gamma photons. Cobalt 60 has a half-life of 5.27 years, and its presence is shown by 1.173-MeV and 1.333-MeV gamma photons. Not more than 1% of the total radioactivity is due to  $^{60}\text{Co}$ ; and not more than 2% of the total radioactivity is due to  $^{58}\text{Co}$ ,  $^{60}\text{Co}$ , and other radionuclidic impurities.

**Content of cyanocobalamin**—Determine the content, in  $\mu\text{g}$  per mL, of cyanocobalamin as directed under *Vitamin B<sub>12</sub> Activity Assay* (171).

#### Change to read:

**Assay for radioactivity**—Using a suitable counting assembly (CN 1-May-2017), determine the radioactivity, in MBq ( $\mu\text{Ci}$ ) per mL, of Oral Solution by use of a calibrated system as directed under *Radioactivity* (821).

## Cyanocobalamin Co 58 Capsules

Vitamin B<sub>12</sub>- $^{58}\text{Co}$

» Cyanocobalamin Co 58 Capsules contain Cyanocobalamin in which a portion of the molecules contain radioactive cobalt ( $^{58}\text{Co}$ ) in the molecular structure. Each Capsule contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $^{58}\text{Co}$  as cyanocobalamin expressed in megabecquerels (or microcuries) at the time indicated in the labeling. The cyanocobalamin content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount.

**Specific activity:** not less than 0.02 MBq (or 0.5  $\mu\text{Ci}$ ) per  $\mu\text{g}$  of cyanocobalamin.

**Packaging and storage**—Preserve in well-closed, light-resistant containers, and store in a cold place.

**Labeling**—Label it to include the following: the date of calibration; the amount of cyanocobalamin expressed in  $\mu\text{g}$  per Capsule; the amount of  $^{58}\text{Co}$  as cyanocobalamin expressed in MBq (or  $\mu\text{Ci}$ ) per Capsule at the time of calibration; the expiration date; and the statement "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay, and also indicates that the radioactive half-life of  $^{58}\text{Co}$  is 70.9 days.

#### USP Reference standards (11)—

USP Cyanocobalamin RS

**Disintegration** (701): 30 minutes, testing one Capsule in 1 N hydrochloric acid maintained at  $37 \pm 2^\circ$  as the immersion fluid.

#### Radionuclide identification (821)—

**A:** Its gamma-ray spectrum is identical to that of a specimen of  $^{58}\text{Co}$  that exhibits major photopeaks at 0.511 MeV (annihilation radiation) and 0.811 MeV.

**B:** The retention time of the major peak in the radiochromatogram of the *Test solution* corresponds to that in the chromatogram of the *Standard solution*, as obtained in the test for *Radiochemical purity*.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Determine the instrument response of each of 10 Capsules by measurement in a suitable counting assembly and under identical geometric conditions. Calculate the average radioactivity per Capsule. The radioactivities of none of the Capsules differ by more than 10% from the average. The relative standard deviation is less than 3.5%.

#### Radiochemical purity—

**Mobile phase**—Prepare a solution of 10.0 g of dibasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of 3.5. Prepare a mixture of the solution so obtained and methanol (73.5:26.5), mix, and degas. Use within 2 days. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard solution**—Transfer about 10 mg of USP Cyanocobalamin RS, accurately weighed, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 2.0 mL of the solution so obtained to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Test solution**—Dissolve the contents of one Capsule in 1 mL of water, allow to stand for about 10 minutes, and centrifuge. Use the supernatant.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 361-nm detector, a gamma detector adjusted for  $^{58}\text{Co}$ , and a 4.6-mm  $\times$  25-cm stainless steel column that contains 5- $\mu\text{m}$  packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*.

**Procedure**—Inject about 100  $\mu\text{L}$  of the *Standard solution* into the chromatograph, record the chromatogram for 30 minutes, and note the retention time of the cyanocobalamin peak. Inject 100  $\mu\text{L}$  of the *Test solution* into the chromatograph, record the chromatogram for three times the retention time of cyanocobalamin, and measure the peak areas using the gamma detector. Calculate the percentage of cyanocobalamin present as cyanocobalamin  $^{58}\text{Co}$  in the portion of Capsules taken by the formula:

$$100(r_u / r_s)$$

in which  $r_u$  is the peak area for cyanocobalamin  $^{58}\text{Co}$  obtained from the *Test solution*; and  $r_s$  is the sum of all the peak areas in the radiochromatogram obtained from the *Test solution*: not less than 90% of the total radioactivity is found as cyanocobalamin  $^{58}\text{Co}$ .

**Radionuclidic purity**—Using a suitable, calibrated instrument (see *Radioactivity* (821)) and standardized solutions of  $^{58}\text{Co}$ ,  $^{57}\text{Co}$ , and  $^{60}\text{Co}$ , record the gamma spectrum. The spectrum does not differ significantly from that of the standardized  $^{58}\text{Co}$  solution. Determine the relative amounts of  $^{58}\text{Co}$ ,  $^{57}\text{Co}$ , and  $^{60}\text{Co}$  present. Cobalt 57 has a half-life of 270.9 days, and its presence is shown by 0.122 MeV gamma photons. Cobalt 60 has a half-life of 5.27 years and its presence is shown by 1.173 MeV and 1.333 MeV gamma photons. Not more than 1% of the total radioactivity is due



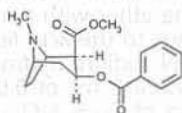
to  $^{60}\text{Co}$ ; and not more than 2% of the total radioactivity is due to  $^{57}\text{Co}$ ,  $^{60}\text{Co}$ , and other radionuclidic impurities.

**Content of cyanocobalamin**—Determine the content, in  $\mu\text{g}$  per Capsule, of cyanocobalamin as directed under *Vitamin B<sub>12</sub> Activity Assay* (171).

#### Change to read:

**Assay for radioactivity** (see *Radioactivity* (821)) (CN 1-May-2017)—Using a suitable counting assembly (CN 1-May-2017) and calibrated system, determine the radioactivity, in MBq (or  $\mu\text{Ci}$ ) per Capsule, of Cyanocobalamin Co 58 Capsules.

## Cocaine



$\text{C}_{17}\text{H}_{21}\text{NO}_4$  303.35

8-Azabicyclo[3.2.1]octane-2-carboxylic acid, 3-(benzoyloxy)-8-methyl-, methyl ester, [1*R*-(*exo*,*exo*)]-.

Methyl 3 $\beta$ -hydroxy-1 $\alpha$ H,5 $\alpha$ H-tropane-2 $\beta$ -carboxylate benzoate (ester) [50-36-2].

» Cocaine, dried over phosphorus pentoxide for 3 hours, contains not less than 99.0 percent and not more than 101.0 percent of  $\text{C}_{17}\text{H}_{21}\text{NO}_4$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

#### USP Reference standards (11)—

USP Cocaine Hydrochloride RS

#### Identification—

**A:** *Ultraviolet Absorption* (197U)—

*Solution:* 15  $\mu\text{g}$  per mL.

*Medium:* dilute hydrochloric acid (1 in 120).

Absorptivities at 233 nm, calculated on the dried basis, do not differ by more than 3.0%.

**B:** It meets the requirements under *Identification—Organic Nitrogenous Bases* (181), USP Cocaine Hydrochloride RS being used, and sodium carbonate TS being used in place of sodium hydroxide TS.

**C:** Dissolve about 100 mg in a mixture of 0.4 mL of dilute hydrochloric acid (1 in 12) and water to make 5 mL, and add 5 drops of chromium trioxide solution (1 in 20): a yellow precipitate is formed, and it quickly redissolves when the mixture is shaken. Add 1 mL of hydrochloric acid: a permanent, orange-colored, crystalline precipitate is formed.

**D:** Dissolve about 10 mg in 1 mL of dilute hydrochloric acid (1 in 600), and evaporate on a steam bath just to dryness. Dissolve the residue in 2 drops of water, and add 1 mL of potassium permanganate solution (1 in 300): a violet, crystalline precipitate is formed, and it appears brownish violet when collected on a filter, and shows characteristic violet-red crystalline aggregates under the low power of a microscope, similar to those obtained from USP Cocaine Hydrochloride RS.

**Melting range, Class I** (741): between 96° and 98°.

**Loss on drying** (731)—Dry it over phosphorus pentoxide for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Readily carbonizable substances** (271)—Dissolve about 500 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid A*.

**Limit of cinnamyl-cocaine and other reducing substances**—Dissolve about 300 mg of finely powdered Cocaine in 1 mL of dilute hydrochloric acid (1 in 12) with the aid of heat, if necessary, and dilute with water to 15 mL. Mix 5 mL of this solution with 0.3 mL of dilute sulfuric acid (1 in 35) and 0.1 mL of potassium permanganate solution (1 in 300): the violet color does not disappear entirely within 30 minutes.

**Limit of isotropyl-cocaine**—Dilute in a beaker 5 mL of the solution of Cocaine prepared in the test for *Cinnamyl-cocaine and other reducing substances* with 80 mL of water, add 0.2 mL of 6 N ammonium hydroxide, and stir the solution vigorously for 5 minutes, occasionally rubbing the inner wall of the beaker with a stirring rod: a crystalline precipitate of cocaine is formed, and the supernatant is clear.

**Assay**—Dissolve about 600 mg of Cocaine, previously dried and accurately weighed, in 50 mL of glacial acetic acid, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 30.34 mg of  $\text{C}_{17}\text{H}_{21}\text{NO}_4$ .

## Cocaine Hydrochloride

$\text{C}_{17}\text{H}_{21}\text{NO}_4 \cdot \text{HCl}$  339.81

8-Azabicyclo[3.2.1]octane-2-carboxylic acid, 3-(benzoyloxy)-8-methyl-, methyl ester, hydrochloride, 1*R*-(*exo*,*exo*)-.

Methyl 3 $\beta$ -hydroxy-1 $\alpha$ H,5 $\alpha$ H-tropane-2 $\beta$ -carboxylate, benzoate (ester) hydrochloride [53-21-4].

» Cocaine Hydrochloride contains not less than 99.0 percent and not more than 101.0 percent of  $\text{C}_{17}\text{H}_{21}\text{NO}_4 \cdot \text{HCl}$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

#### USP Reference standards (11)—

USP Cocaine Hydrochloride RS

#### Identification—

**A:** It meets the requirements under *Identification—Organic Nitrogenous Bases* (181), sodium carbonate TS being used in place of 1 N sodium hydroxide.

**B:** To 5 mL of a solution (1 in 50) add 5 drops of chromium trioxide solution (1 in 20): a yellow precipitate is formed, and it quickly redissolves when the mixture is shaken gently. Add 1 mL of hydrochloric acid: a permanent, orange-colored crystalline precipitate is formed.

**C:** To a solution of about 10 mg in 2 drops of water add 1 mL of 0.1 N potassium permanganate: a violet, crystalline precipitate is formed, and it appears brownish violet when collected on a filter, and shows characteristic, violet-red crystalline aggregates under the low power of a microscope.

**D:** It responds to the tests for *Chloride* (191).

**Specific rotation** (781S): between  $-71^\circ$  and  $-73^\circ$ .

*Test solution:* 20 mg, previously dried, per mL, in water.

**Acidity**—Dissolve 500 mg in 10 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide: not more than 0.50 mL is required to produce a yellow color.

**Loss on drying** (731)—Dry it over silica gel for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Readily carbonizable substances** (271)—Dissolve 500 mg in 5 mL of sulfuric acid: the solution has no more color than *Matching Fluid F*.

**Limit of cinnamyl-cocaine and other reducing substances**—To 5 mL of a solution (1 in 50) add 0.3 mL of 1 N sulfuric acid and 0.10 mL of 0.10 N potassium permanga-



nate: the violet color does not disappear entirely within 30 minutes.

**Limit of isotropyl-cocaine**—Dilute 5 mL of a solution (1 in 50) in a beaker with 80 mL of water, add 0.2 mL of 6 N ammonium hydroxide, stir the solution vigorously during 5 minutes, occasionally rubbing the inner wall of the beaker with a stirring rod: a crystalline precipitate of cocaine is formed, and the supernatant is clear.

**Assay**—Dissolve about 500 mg of Cocaine Hydrochloride, accurately weighed, in a mixture of 40 mL of glacial acetic acid and 10 mL of mercuric acetate TS. Add 2 drops of quinaldine red TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 33.98 mg of  $C_{17}H_{21}NO_4 \cdot HCl$ .

### Cocaine Hydrochloride Tablets for Topical Solution

» Cocaine Hydrochloride Tablets for Topical Solution contain not less than 91.0 percent and not more than 109.0 percent of the labeled amount of  $C_{17}H_{21}NO_4 \cdot HCl$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Cocaine Hydrochloride RS

**Identification**—

A: Add 5 drops of chromium trioxide solution (1 in 20) to 5 mL of a filtered solution of Tablets, equivalent to cocaine hydrochloride solution (1 in 50): a yellow precipitate is formed and it redissolves when the mixture is shaken. On the addition of 1 mL of hydrochloric acid, a permanent, yellowish orange, crystalline precipitate is formed.

B: Dissolve a portion of powdered Tablets, equivalent to about 10 mg of cocaine hydrochloride, in 1 mL of water, filter, and add 2 mL of 0.1 N potassium permanganate: a red-purple, crystalline precipitate, which appears brown when collected on a filter, is formed, and it shows characteristic, crystalline aggregates under the low power of a microscope.

C: Add silver nitrate TS, dropwise, to a filtered solution of Tablets, equivalent to cocaine hydrochloride solution (1 in 20): a white precipitate is formed, and it is insoluble in nitric acid.

**Disintegration** (701): 15 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—Place 1 Tablet in a 100-mL volumetric flask, add 50 mL of water, and shake the flask until the tablet is dissolved. Dilute with water to volume, mix, and filter, discarding the first 20 mL of the filtrate. Dilute a portion of the subsequent filtrate, if necessary, with water to provide a solution containing approximately 80 µg of cocaine hydrochloride per mL. Concomitantly determine the absorbances of this test solution and a Standard solution of USP Cocaine Hydrochloride RS in the same medium having a known concentration of about 80 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 275 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $C_{17}H_{21}NO_4 \cdot HCl$  in the Tablet by the formula:

$$(T/D)C(A_U/A_S)$$

in which  $T$  is the labeled quantity, in mg, of cocaine hydrochloride in the Tablet;  $D$  is the concentration, in µg per mL,

of cocaine hydrochloride in the test solution, based upon the labeled quantity per Tablet and the extent of dilution;  $C$  is the concentration, in µg per mL, of USP Cocaine Hydrochloride RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Tablet and the Standard solution, respectively.

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Dissolve an accurately weighed portion of the powder, equivalent to about 60 mg of cocaine hydrochloride, in 10 mL of water, render the solution slightly alkaline with 6 N ammonium hydroxide, and completely extract the cocaine with small successive portions of ether. Evaporate the combined ether extracts on a steam bath to one-half their volume, transfer the remaining liquid to a separator, and wash with three 5-mL portions of water. Shake the water washings with a small portion of ether, and add the ether washing to the combined ether extracts. Add 10.0 mL of 0.05 N sulfuric acid VS to the ether solution, agitate the mixture thoroughly, and draw off the acidified water layer into a beaker. Wash the ether with two small portions of water, add the washings to the acid liquid, and titrate the excess acid with 0.02 N sodium hydroxide VS, using methyl red TS as the indicator. Each mL of 0.05 N sulfuric acid is equivalent to 16.99 mg of  $C_{17}H_{21}NO_4 \cdot HCl$ .

### Cocaine and Tetracaine Hydrochlorides and Epinephrine Topical Solution

#### DEFINITION

Cocaine and Tetracaine Hydrochlorides and Epinephrine Topical Solution contains NLT 3.6 g and NMT 4.4 g of cocaine hydrochloride ( $C_{17}H_{21}NO_4 \cdot HCl$ ), NLT 0.90 g and NMT 1.10 g of tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ), and NLT 20 mg and NMT 30 mg of epinephrine ( $C_9H_{13}NO_3$ ) in each 100 mL of Topical Solution.

Prepare Cocaine and Tetracaine Hydrochlorides and Epinephrine Topical Solution as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Cocaine Hydrochloride	4.0 g
Tetracaine Hydrochloride	1.0 g
Epinephrine Injection (1:1000)	25.0 mL
Benzalkonium Chloride	10 mg
Edetate Disodium	6.4 mg
Sodium Chloride Injection (0.9%)	35 mL
Purified Water, a sufficient quantity to make	100 mL

Dissolve the Cocaine Hydrochloride and Tetracaine Hydrochloride in 25 mL of Purified Water, and add the Epinephrine Injection (1:1000). Separately dissolve Edetate Disodium in Sodium Chloride Injection (0.9%), and dilute quantitatively and stepwise, if necessary, with Sodium Chloride Injection (0.9%) to obtain 35 mL of a solution containing 6.4 mg of Edetate Disodium. Similarly, and separately, dissolve Benzalkonium Chloride in Purified Water (or use Benzalkonium Chloride Solution), and dilute quantitatively and stepwise, if necessary, with Purified Water to obtain 10 mL of a solution containing 10 mg of Benzalkonium Chloride. Combine the three solutions, add sufficient Purified Water to bring to final volume, and mix well.

#### ASSAY

##### • TETRACAINE HYDROCHLORIDE

**Solution A:** 6.3 g/L of monobasic potassium phosphate containing 0.55 g/L of sodium 1-octanesulfonate. Adjust with phosphoric acid to a pH of 2.5.

**Solution B:** Acetonitrile and Solution A (10:90). Pass through a suitable filter of 0.5-µm or finer pore size, and degas.



**Solution C:** Acetonitrile and *Solution A* (30:70). Pass through a suitable filter of 0.5- $\mu$ m or finer pore size, and degas.

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution B (%)	Solution C (%)
0	100	0
5	100	0
10	0	100
24	0	100
25	100	0
75	100	0

**Standard solution:** 0.5 mg/mL of USP Tetracaine Hydrochloride RS

**Sample solution:** Transfer 0.5 mL of Topical Solution to a 10-mL volumetric flask, dilute with water to volume, and mix.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 3.9-mm  $\times$  30-cm; 10- $\mu$ m packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 1.5 for the analyte peak

**Relative standard deviation:** NMT 2.0% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in g, of tetracaine hydrochloride ( $C_{15}H_{24}N_2O_2 \cdot HCl$ ) in 100 mL of the Topical Solution:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times F$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of tetracaine hydrochloride in the *Standard solution* (mg/mL)

$D$  = dilution factor, 20

$V$  = final volume of Topical Solution, 100 mL

$F$  = conversion factor,  $10^{-3}$  g/mg

**Acceptance criteria:** 0.90–1.10 g

#### • COCAINE HYDROCHLORIDE

**Solution A, Solution B, Solution C, Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the Assay for *Tetracaine Hydrochloride*.

**Standard solution:** 2 mg/mL of USP Cocaine Hydrochloride RS

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the quantity, in g, of cocaine hydrochloride ( $C_{17}H_{21}NO_4 \cdot HCl$ ) in 100 mL of the Topical Solution:

$$\text{Result} = (r_U/r_S) \times C_S \times D \times V \times F$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of cocaine hydrochloride in the *Standard solution* (mg/mL)

$D$  = dilution factor, 20

$V$  = final volume of Topical Solution, 100 mL

$F$  = conversion factor,  $10^{-3}$  g/mg

**Acceptance criteria:** 3.6–4.4 g

#### • EPINEPHRINE

**Solution A, Solution B, Solution C, Mobile phase, Sample solution, and Chromatographic system:** Pro-

ceed as directed in the Assay for *Tetracaine Hydrochloride*.

**Standard solution:** Transfer 3 mg of USP Epinephrine Bitartrate RS to a 25-mL volumetric flask, and dilute with water to volume. Transfer 4.0 mL of the resultant solution to a 25-mL volumetric flask, and dilute with water to volume.

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

Calculate the quantity, in mg, of epinephrine ( $C_9H_{13}NO_3$ ) in 100 mL of the Topical Solution:

$$\text{Result} = (r_U/r_S) \times C_S \times (M_{r1}/M_{r2}) \times D \times V$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of epinephrine bitartrate in the *Standard solution* (mg/mL)

$M_{r1}$  = molecular weight of epinephrine, 183.20

$M_{r2}$  = molecular weight of epinephrine bitartrate, 333.29

$D$  = dilution factor, 20

$V$  = final volume of Topical Solution, 100 mL

**Acceptance criteria:** 20–30 mg

#### SPECIFIC TESTS

- **pH (791):** 4.0–6.0

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in sterile, tight, light-resistant containers. Store in a refrigerator.
- **LABELING:** Label it to indicate that it is intended for external use only and that it is not to be used if a precipitate is present. The label states that it is to be protected from light.
- **BEYOND-USE DATE:** NMT 30 days after the date on which it was compounded
- **USP REFERENCE STANDARDS (11)**
  - USP Cocaine Hydrochloride RS
  - USP Epinephrine Bitartrate RS
  - USP Tetracaine Hydrochloride RS

## Cod Liver Oil

#### DEFINITION

Cod Liver Oil is the partially destearinated fixed oil obtained from fresh livers of *Gadus morrhua* L. and other species of Fam. Gadidae. Cod Liver Oil contains, in each g, NLT 180  $\mu$ g (600 USP Units) and NMT 750  $\mu$ g (2500 USP Units) of vitamin A and NLT 1.5  $\mu$ g (60 USP Units) and NMT 6.25  $\mu$ g (250 USP Units) of vitamin D.

Cod Liver Oil may be flavored by the addition of NMT 1% of a suitable flavor or a mixture of flavors. A suitable antioxidant may be added.

#### IDENTIFICATION

##### • A. PRESENCE OF VITAMIN A

**Sample solution:** 25 mg/mL of Cod Liver Oil in chloroform

**Analysis:** To 1 mL of the *Sample solution* add 10 mL of antimony trichloride TS.

**Acceptance criteria:** A blue color results immediately.

##### • B. FATTY ACID PROFILE

**Antioxidant solution:** 0.05 mg/mL of butylated hydroxytoluene in hexanes

**System suitability solution:** Prepare a mixture containing equal amounts of methyl palmitate, methyl stearate, methyl arachidate, and methyl behenate in *Antioxidant solution*.

**Standard stock solution:** 45 mg/mL of USP Cod Liver Oil RS in *Antioxidant solution*



**Standard solution:** Transfer 2.0 mL of the *Standard stock solution* into a quartz tube, and evaporate with a gentle stream of nitrogen. Add 1.5 mL of a 2% solution of sodium hydroxide in methanol, cap tightly with a polytetrafluoroethylene-lined cap, mix, and heat in a water bath for 7 min. Cool, add 2 mL of a 120 mg/mL solution of boron trichloride in methanol, cover with nitrogen, cap tightly, mix, and heat in a water bath for 30 min. Cool to 40°–50°, add 1 mL of isooctane, cap, and mix in a vortex mixer or shake vigorously for at least 30 s. Immediately add 5 mL of saturated sodium chloride solution, cover with nitrogen, cap, and mix in a vortex mixer or shake thoroughly for at least 15 s. Allow the upper layer to become clear, and transfer to a separate tube. Shake the methanol layer once more with 1 mL of isooctane, and combine the isooctane extracts. Wash the combined extracts twice with 1 mL of water, and dry over anhydrous sodium sulfate.

**Sample solution:** Proceed as directed for the *Standard solution*, except replace USP Cod Liver Oil RS with Cod Liver Oil.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m fused silica capillary column bonded with a 0.25-μm film of phase G16

Temperature

Injector: 250°

Detector: 280°

Column: See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	1	225	20

Carrier gas: Helium

Split flow ratio: 200:1

Injection size: 1 μL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*.

#### Suitability requirements

**Chromatogram similarity:** The chromatogram from the *Standard solution* is similar to the reference chromatogram supplied with USP Cod Liver Oil RS. Identify the retention times of the relevant fatty acid methyl esters by comparing the chromatogram of the *Standard solution* with the reference chromatogram supplied with USP Cod Liver Oil RS.

**Resolution:** NLT 1.3 between methyl oleate and methyl *cis*-vaccinate, and that between methyl gadoleate and methyl gondoate is sufficient for purposes of identification and area measurement, *Standard solution*.

**Theoretical area percentages:** 24.4 ± 1 for methyl palmitate, 24.8 ± 1 for methyl stearate, 25.2 ± 1 for methyl arachidate, and 25.6 ± 1 for methyl behenate, *System suitability solution*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Identify the retention times of the relevant fatty acid methyl esters in the *Sample solution* by comparing the chromatogram of the *Sample solution* with that of the *Standard solution*.

Determine the number of fatty acid methyl esters in the *Sample solution*. The number of fatty acid methyl ester peaks exceeding 0.05% of the total area of fatty acid methyl esters is at least 24, and the 24 largest peaks of the methyl esters account for more than 90% of the total area. (These correspond to the following, in com-

mon elution order: 14:0, 15:0, 16:0, 16:1 *n*-7, 16:4 *n*-1, 18:0, 18:1 *n*-9, 18:1 *n*-7, 18:2 *n*-6, 18:3 *n*-3, 18:4 *n*-3, 20:1 *n*-11, 20:1 *n*-9, 20:1 *n*-7, 20:2 *n*-6, 20:4 *n*-6, 20:3 *n*-3, 20:4 *n*-3, 20:5 *n*-3, 22:1 *n*-11, 22:1 *n*-9, 21:5 *n*-3, 22:5 *n*-3, and 22:6 *n*-3.)

Calculate the area percentage for each fatty acid methyl ester in the portion of Cod Liver Oil taken:

$$\text{Result} = (r_A/r_B) \times 100$$

$r_A$  = peak area of each individual fatty acid methyl ester

$r_B$  = total area from all peaks, except the solvent peak and butylated hydroxytoluene

**Acceptance criteria:** The *Sample solution* meets the limits described in Table 2.

Table 2

Fatty Acid	Shorthand Notation	Lower Limit (Area %)	Upper Limit (Area %)
<b>Saturated fatty acids</b>			
Myristic acid	14:0	2.0	6.0
Palmitic acid	16:0	7.0	14.0
Stearic acid	18:0	1.0	4.0
<b>Monounsaturated fatty acids</b>			
Palmitoleic acid	16:1 <i>n</i> -7	4.5	11.5
<i>cis</i> -Vaccenic acid	18:1 <i>n</i> -7	2.0	7.0
Oleic acid	18:1 <i>n</i> -9	12.0	21.0
Gadoleic acid	20:1 <i>n</i> -11	1.0	5.5
Gondoic acid	20:1 <i>n</i> -9	5.0	17.0
Erucic acid	22:1 <i>n</i> -9	0	1.5
Cetoleic acid	22:1 <i>n</i> -11	5.0	12.0
<b>Polyunsaturated fatty acids</b>			
Linoleic acid	18:2 <i>n</i> -6	0.5	3.0
$\alpha$ -Linolenic acid	18:3 <i>n</i> -3	0	2.0
Morotic acid	18:4 <i>n</i> -3	0.5	4.5
Eicosapentaenoic acid	20:5 <i>n</i> -3	7.0	16.0
Docosahexaenoic acid	22:6 <i>n</i> -3	6.0	18.0

#### ASSAY

##### • VITAMIN A

**Sample:** 500 mg to 1 g of Cod Liver Oil

**Analysis:** Proceed as directed under *Vitamin A Assay* (571).

**Acceptance criteria:** 180 μg (600 USP Units) to 750 μg (2500 USP Units) of vitamin A per g of Cod Liver Oil

##### • VITAMIN D

**Solution A:** *n*-Amyl alcohol and dehydrated hexane (3:997)

**Solution B:** Acetonitrile, water, and phosphoric acid (96:3.8:0.2)

**Butylated hydroxytoluene solution:** 10 mg/mL of butylated hydroxytoluene in chromatographic hexane

**Aqueous potassium hydroxide solution:** Dissolve 800 g of potassium hydroxide in 1000 mL of freshly boiled water, mix, and cool. [NOTE—Prepare this solution fresh daily.]

**Alcoholic potassium hydroxide solution:** Dissolve 3 g of potassium hydroxide in 50 mL of freshly boiled water, add 10 mL of alcohol, and dilute with freshly boiled water to 100 mL. [NOTE—Prepare this solution fresh daily.]

**Ascorbic acid solution:** 100 mg/mL of ascorbic acid in water. [NOTE—Prepare this solution fresh daily.]

**Internal standard solution:** 5 μg/mL of USP Ergocalciferol RS in alcohol

**Standard stock solution:** 5 μg/mL of USP Cholecalciferol RS in alcohol



**Standard solution:** Transfer 2.0 mL of the *Standard stock solution* and 2.0 mL of the *Internal standard solution* to a round-bottomed flask. Proceed as directed for *Sample solution 1* beginning with "Add 5 mL of..."

**Sample solution 1:** Transfer 4.00 g of Cod Liver Oil to a round-bottomed flask. Add 5 mL of *Ascorbic acid solution*, 100 mL of alcohol, and 10 mL of *Aqueous potassium hydroxide solution*, and mix. Reflux the mixture on a steam bath for 30 min. Add 100 mL of a 10 mg/mL sodium chloride solution. Cool rapidly under running water, and transfer the saponified mixture to a 500-mL separator, rinsing the saponification flask with 75 mL of a 10 mg/mL sodium chloride solution, and then with 150 mL of a mixture of ether and hexane (1:1). Shake the combined saponified mixture and rinsings vigorously for 30 s, and allow to stand until both layers are clear. Discard the lower layer. Wash the ether-hexane extracts by shaking vigorously with 50 mL of *Alcoholic potassium hydroxide solution*, and then washing with three 50-mL portions of a 10 mg/mL sodium chloride solution. Filter the upper layer through 5 g of anhydrous sodium sulfate on a fast filter paper into a 250-mL flask suitable for a rotary evaporator. Wash the filter with 10 mL of a mixture of ether and hexane (1:1), and combine with the extract. Evaporate the solvent at reduced pressure at a temperature not exceeding 30°, and fill with nitrogen when the evaporation is complete. Alternatively evaporate the solvent under a gentle stream of nitrogen at a temperature not exceeding 30°. Dissolve the residue in 1.5 mL of *Butylated hydroxytoluene solution*. [NOTE—Gentle heating in an ultrasonic bath may be required. A large fraction of the white residue is cholesterol.]

**Sample solution 2:** To 4.00 g of Cod Liver Oil add 2.0 mL of *Internal standard solution*, and proceed as directed for *Sample solution 1* beginning with "Add 5 mL of..."

#### Clean-up chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Mobile phase: *Solution A*

Clean-up column: 4.6-mm × 25-cm stainless steel; packing L10

Injection size: 350 µL

#### Analysis (clean-up)

Samples: *Standard solution*, *Sample solution 1*, and *Sample solution 2*

Collect separately the eluates from 2 min before to 2 min after the retention time of cholecalciferol in a glass tube containing 1 mL of *Butylated hydroxytoluene solution* and fitted with a hermetic closure. Evaporate each tube under a stream of nitrogen at a temperature not exceeding 30°. Dissolve each residue in 1.5 mL of acetonitrile, and inject into the analytical chromatographic system below.

#### Analytical chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: UV 265 nm

Mobile phase: *Solution B*

Analytical column: 4.6-mm × 15-cm stainless steel; 5-µm packing L1

Injection size: 200 µL

#### System suitability

Sample: *Standard solution* (after the clean-up)

#### Suitability requirements

Resolution: NLT 1.4 between cholecalciferol and ergocalciferol

Relative standard deviation: NMT 2.0% for the cholecalciferol peak from replicate injections

#### Analysis

Samples: *Standard solution*, *Sample solution 1*, and *Sample solution 2* (after the clean-up)

Calculate the content of vitamin D, in µg/g, in the portion of Cod Liver Oil taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

$R_U$  = peak response of cholecalciferol relative to the corrected internal standard in the *Sample solution 2*, as calculated below

$R_S$  = peak response of cholecalciferol relative to the internal standard in the *Standard solution*

$C_S$  = concentration of USP Cholecalciferol RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Cod Liver Oil in *Sample solution 2* (g/mL)

$$R_U = r_{U2}/[r_{I2} - (r_{I1} \times r_{U2}/r_{U1})]$$

$r_{U2}$  = peak response for cholecalciferol from *Sample solution 2*

$r_{I2}$  = peak response for the internal standard from *Sample solution 2*

$r_{I1}$  = peak response for the internal standard from *Sample solution 1*

$r_{U1}$  = peak response for cholecalciferol from the *Sample solution 1*

**Acceptance criteria:** 1.5 µg (60 USP Units) to 6.25 µg (250 USP Units) of vitamin D per g of Cod Liver Oil

#### SPECIFIC TESTS

• **SPECIFIC GRAVITY** <841>: 0.918–0.927

• **COLOR:** When viewed transversely in a tall, cylindrical, standard oil-specimen bottle of colorless glass of about 120-mL capacity, the color of the Cod Liver Oil is not more intense than that of a mixture of cobaltous chloride CS, ferric chloride CS, and water (11:76:33), in a similar bottle of the same internal diameter.

• **NONDESTEARINATED COD LIVER OIL**

Sample: Cod Liver Oil

Analysis: Fill a tall, cylindrical, standard oil-specimen bottle of 120-mL capacity with the *Sample* at a temperature between 23° and 28°, insert the stopper, and immerse the bottle in a mixture of ice and water for 3 h.

Acceptance criteria: The oil remains clear and does not deposit stearin.

• **FATS AND FIXED OILS, Unsaponifiable Matter** <401>: NMT 1.30%

• **FATS AND FIXED OILS, Acid Value** <401>

Sample solution: Mix 15 mL of alcohol with 15 mL of ether, add 5 drops of phenolphthalein TS, and neutralize with 0.1 N sodium hydroxide. Dissolve 2.0 g of Cod Liver Oil in the mixture, and boil the oil solution gently under a reflux condenser for 10 min.

Analysis: Cool, and titrate the mixture with 0.1 N sodium hydroxide VS to the production of a pink color that persists after shaking for 30 s.

Acceptance criteria: NMT 1.0 mL of 0.1 N sodium hydroxide is required.

• **FATS AND FIXED OILS, Iodine Value** <401>: 145–180

• **FATS AND FIXED OILS, Saponification Value** <401>: 180–192

[NOTE—If carbon dioxide has been used as a preservative, expose the Cod Liver Oil in a shallow dish in a vacuum desiccator for 24 h before weighing the specimen for determination of the saponification value.]

• **FATS AND FIXED OILS, Anisidine Value** <401>: NMT 30

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. It may be bottled or otherwise packaged in containers from which air has been expelled by the production of a vacuum or by an inert gas.

• **LABELING:** The vitamin A potency and vitamin D potency, when designated on the label, are expressed in USP Units/g of oil. The potencies may be expressed also in metric units, on the basis that 1 USP Vitamin A Unit equals 0.3 µg and 40 USP Vitamin D Units equals 1 µg. Where the content of docosahexaenoic acid or eicosa-



pentaenoic acid is claimed, state the concentration in mg/g.

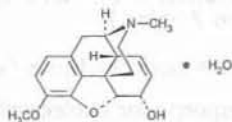
• **USP Reference Standards** (11)

USP Cholecalciferol RS

USP Cod Liver Oil RS

USP Ergocalciferol RS

## Codeine



$C_{18}H_{21}NO_3 \cdot H_2O$  317.38

Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-, monohydrate, (5 $\alpha$ ,6 $\alpha$ )-.

7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -ol monohydrate [6059-47-8].

Anhydrous 299.37 [76-57-3].

» Codeine, dried at 80° for 4 hours, contains not less than 98.5 percent and not more than 100.5 percent of  $C_{18}H_{21}NO_3$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Codeine Sulfate RS

**Identification**—

**A: Infrared Absorption** (197K)—Proceed as directed with the codeine test specimen and the codeine standard specimen obtained from 50 mg of USP Codeine Sulfate RS dissolved in 15 mL of water, then rendered alkaline with 6 N ammonium hydroxide and extracted with several 10-mL portions of chloroform, followed by evaporation of the combined chloroform extracts on a steam bath to dryness, and drying at 80° for 4 hours.

**B: Ultraviolet Absorption** (197U)—

**Solution:** 100  $\mu$ g per mL.

**Medium:** 0.1 N sulfuric acid.

Absorptivity at 284 nm, calculated on the dried basis, is between 112.9% and 119.9% of that of USP Codeine Sulfate RS.

**Melting range** (741)—When previously dried, it melts between 154° and 158°, but the range between beginning and end of melting does not exceed 2°.

**Loss on drying** (731)—Dry it at 80° for 4 hours: it loses not more than 6.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Readily carbonizable substances** (271)—Dissolve 10 mg in 5 mL of sulfuric acid: the solution has no more color than Matching Fluid S.

**Chromatographic purity**—Prepare a solution of it in dehydrated alcohol containing 40 mg per mL (*Solution A*). Dilute 2.0 mL of *Solution A* with dehydrated alcohol to 100 mL (*Solution B*). Dilute 1.0 mL of *Solution A* with dehydrated alcohol to 100 mL (*Solution C*). Apply separate 10- $\mu$ L volumes of *Solution A*, *Solution B*, and *Solution C* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of dehydrated alcohol, cyclohexane, and ammonium hydroxide (72:30:6) until the solvent front has moved three-fourths of the length of the plate. Remove the plate from the developing chamber, and allow the solvent to evaporate. Spray the plate with a

reagent prepared by mixing 3 mL of chloroplatinic acid solution (1 in 10) with 97 mL of water, followed by the addition of 100 mL of potassium iodide solution (6 in 100), and examine the chromatogram: no spot obtained from *Solution A*, other than the principal spot and any spot observed at the origin, is more intense than the principal spot obtained from *Solution B* (2%); and not more than one such spot having an  $R_f$  greater than that of the principal spot is more intense than the principal spot obtained from *Solution C* (1%).

**Limit of morphine**—Dissolve about 50 mg of potassium ferricyanide in 10 mL of water, and add 1 drop of ferric chloride TS and 1 mL of a neutral or slightly acid solution of Codeine (1 in 100) prepared with the aid of sulfuric acid: no blue color is produced immediately.

**Assay**—Dissolve about 400 mg of Codeine, previously dried and accurately weighed, by warming it in 30.0 mL of 0.1 N sulfuric acid VS. Cool, and add 10 mL of water. Add methyl red TS, and titrate the excess acid with 0.1 N sodium hydroxide VS. Perform a blank determination (see *Residual Titrations* under *Titrimetry* (541)). Each mL of 0.1 N sulfuric acid is equivalent to 29.94 mg of  $C_{18}H_{21}NO_3$ .

## Codeine Phosphate

$C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$  406.37

Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-

17-methyl-, (5 $\alpha$ ,6 $\alpha$ )-, phosphate (1:1) (salt), hemihydrate.

7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -ol phosphate (1:1) (salt) hemihydrate [41444-62-6].

Anhydrous 397.37 [52-28-8].

» Codeine Phosphate contains not less than 99.0 percent and not more than 101.5 percent of  $C_{18}H_{21}NO_3 \cdot H_3PO_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers. Store up to 40° as permitted by the manufacturer.

**USP Reference standards** (11)—

USP Codeine Phosphate RS

**Identification**—

**A: Infrared Absorption** (197K).

**B:** Neutralize a solution (1 in 50) with 6 N ammonium hydroxide, and add silver nitrate TS: a yellow precipitate of silver phosphate is formed, and it is soluble in 2 N nitric acid and in 6 N ammonium hydroxide.

**Acidity**—Dissolve 100 mg in 20 mL of water, and titrate with 0.010 N sodium hydroxide to a pH of 5.4, using a pH meter: not more than 1.0 mL of 0.010 N sodium hydroxide is required.

**Water Determination, Method I** (921): not more than 3.0%.

**Chloride**—To 10 mL of a solution (1 in 100), acidified with nitric acid, add a few drops of silver nitrate TS: no opalescence is produced immediately.

**Sulfate**—To 10 mL of a solution (1 in 100) add a few drops of barium chloride TS: no turbidity is produced immediately.

**Limit of morphine**—Dissolve about 50 mg of potassium ferricyanide in 10 mL of water, and add 1 drop of ferric chloride TS and 1 mL of a solution of Codeine Phosphate (1 in 100): no blue color is produced immediately.

**Chromatographic purity**—Using Codeine Phosphate, proceed as directed in the test for *Chromatographic purity* under *Codeine*, except to use a mixture of 0.01 N hydrochloric acid and dehydrated alcohol (4:1), instead of dehydrated alcohol, to prepare *Solution A*, *Solution B*, and *Solution C*.



**Assay**—Dissolve about 1 g of Codeine Phosphate, accurately weighed, in 50 mL of glacial acetic acid, warming slightly if necessary to effect solution, and titrate with 0.1 N perchloric acid VS, determining the endpoint potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 39.74 mg of  $C_{18}H_{21}NO_3 \cdot H_3PO_4$ .

## Codeine Phosphate Injection

» Codeine Phosphate Injection is a sterile solution of Codeine Phosphate in Water for Injection. It contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ .

**NOTE**—Do not use the Injection if it is more than slightly discolored or contains a precipitate.

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

### USP Reference standards (11)—

USP Codeine Phosphate RS

USP Endotoxin RS

### Identification—

**A:** Dilute a volume of Injection, equivalent to about 90 mg of codeine phosphate, with water to about 10 mL, add 1 drop of hydrochloric acid, and extract with three 10-mL portions of chloroform, discarding the chloroform extracts. Add 6 N ammonium hydroxide until the solution is alkaline, and extract with several 10-mL portions of chloroform. Evaporate the combined chloroform extracts on a steam bath to dryness, and dry at 80° for 4 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima at the same wavelengths as that of the codeine obtained by similarly treating 1 mL of a solution of USP Codeine Phosphate RS (1 in 100).

**B:** A volume of Injection, equivalent to about 60 mg of codeine phosphate, responds to *Identification test B* under *Codeine Phosphate*.

**Bacterial Endotoxins Test** (85)—It contains not more than 5.8 USP Endotoxin Units per mg of codeine phosphate.

**pH** (791): between 3.0 and 6.0.

**Limit of morphine**—Diluted with water to a concentration of 5 mg of codeine phosphate per mL, it meets the requirements of the test for *Limit of morphine* under *Codeine Phosphate*.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—Transfer an accurately measured volume of Injection, equivalent to about 75 mg of codeine phosphate, to a small separator, and add about 15 mL of water. Add 2 drops of phosphoric acid, and extract with four 10-mL portions of chloroform, collecting the chloroform extracts in a separator. Wash the combined chloroform extracts with 10 mL of water, and add the water wash to the first separator containing the sample. Discard the chloroform extracts. Proceed as directed in the *Assay* under *Codeine Phosphate Tablets*, beginning with "render the solution alkaline with 6 N ammonium hydroxide." Each mL of 0.02 N sulfuric acid is equivalent to 8.128 mg of  $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ .

## Codeine Phosphate Compounded Oral Solution

### DEFINITION

Codeine Phosphate Compounded Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ).

Prepare Codeine Phosphate Compounded Oral Solution 3 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Codeine Phosphate	300 mg
Purified Water	1.3 mL
Vehicle: Ora-Sweet, <sup>a</sup> a sufficient quantity to make	100 mL

<sup>a</sup>Paddock Laboratories, Minneapolis, MN.

Calculate the required quantity of each ingredient for the total amount to be prepared. Place *Codeine Phosphate* powder in a suitable calibrated container. Add *Purified Water* and mix to dissolve. Add the *Ora-Sweet* to bring to final volume, and mix well.

### ASSAY

#### • PROCEDURE

**Buffer:** 25 mM monobasic potassium phosphate adjusted with phosphoric acid to a pH of 2.5

**Mobile phase:** Methanol and *Buffer* (30:70). Filter and degas.

**Internal standard solution:** 0.5 mg/mL of theophylline  
**Standard stock solution:** 1.0 mg/mL of USP Codeine Phosphate RS

**Standard solution:** Pipet 0.5 mL of the *Standard stock solution* into a 100-mL volumetric flask, add 0.6 mL of the *Internal standard solution*, dilute with water to volume, and pass through a membrane filter of 0.2-μm pore size to obtain a solution with nominal concentrations of 5 μg/mL of codeine phosphate and 3 μg/mL of theophylline.

**Sample solution:** Shake the Oral Solution thoroughly by hand. Transfer 2 mL of Oral Solution from each bottle into a 50-mL volumetric flask, and dilute with water to volume. Transfer 4.17 mL of the diluted sample to a 100-mL volumetric flask, add 0.6 mL of *Internal standard solution*, dilute with water to volume, and filter to obtain a solution with nominal concentrations of 5 μg/mL of codeine phosphate and 3 μg/mL of theophylline.

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm × 10-cm; 5-μm packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 μL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention times for codeine phosphate and theophylline are about 1.9 and 3.8 min, respectively.]

#### Suitability requirements

**Relative standard deviation:** NMT 2.0% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of codeine phosphate ( $C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$ ) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$R_U$  = peak response ratio of codeine phosphate to the internal standard from the *Sample solution*



- $R_s$  = peak response ratio of codeine phosphate to the internal standard from the *Standard solution*
- $C_s$  = concentration of USP Codeine Phosphate RS in the *Standard solution* ( $\mu\text{g/mL}$ )
- $C_u$  = nominal concentration of codeine phosphate in the *Sample solution* ( $\mu\text{g/mL}$ )
- $M_{r1}$  = molecular weight of codeine phosphate hemihydrate, 406.37
- $M_{r2}$  = molecular weight of anhydrous codeine phosphate, 397.37
- Acceptance criteria: 90.0%–110.0%

**SPECIFIC TESTS**

- **PH (791):** 3.7–4.7

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded, when stored at controlled room temperature
- **LABELING:** Label it to indicate the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS (11)**  
USP Codeine Phosphate RS

**Codeine Phosphate Tablets**

» Codeine Phosphate Tablets contain not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ .

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards (11)**—  
USP Codeine Phosphate RS

**Identification**

**A:** Digest a quantity of finely powdered Tablets, equivalent to about 100 mg of codeine phosphate, with 15 mL of water and 5 mL of 2 N sulfuric acid for 1 hour. Filter, if necessary, and wash any undissolved residue with a few mL of water. Render the filtrate alkaline with 6 N ammonium hydroxide, extract with several small portions of chloroform, and proceed as directed in *Identification test A* under *Codeine Phosphate Injection*, beginning with "Evaporate the combined chloroform extracts." The specified results are observed.

**B:** To a quantity of finely powdered Tablets, equivalent to about 100 mg of codeine phosphate, add 10 mL of water and 2 drops of 2 N sulfuric acid. Digest, with frequent shaking, for 15 minutes, and filter. Neutralize 5 mL of the filtrate with 6 N ammonium hydroxide, and add silver nitrate TS: a yellow precipitate of silver phosphate is formed, and it is soluble in diluted nitric acid and in 6 N ammonium hydroxide.

**Dissolution (711)**

*Medium:* water; 900 mL.

*Apparatus 2:* 50 rpm.

*Time:* 45 minutes.

**Procedure**—Determine the amount of  $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$  dissolved from UV absorbances at the wavelength of maximum absorbance at about 284 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Codeine Phosphate RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$  is dissolved in 45 minutes.

**Uniformity of dosage units (905):** meet the requirements.

**Procedure for content uniformity**—Transfer 1 Tablet, previously crushed or finely powdered, to a 50-mL volumetric flask, add 25 mL of water, and shake to dissolve. Dilute with water to volume, and filter, if necessary, discarding the first 20 mL of the filtrate. Transfer an aliquot of the filtrate, equivalent to about 6 mg of codeine phosphate, to a 50-mL volumetric flask containing 2 mL of 3 N hydrochloric acid, and dilute with water to volume. Dissolve an accurately weighed quantity of USP Codeine Phosphate RS in 0.1 N hydrochloric acid, and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 120  $\mu\text{g}$  per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 284 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of  $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$  in the Tablet taken by the formula:

$$2.5(C/V)(A_u/A_s)(406.37/397.37)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Codeine Phosphate RS in the Standard solution; V is the volume, in mL, of the aliquot taken of the solution of the Tablet;  $A_u$  and  $A_s$  are the absorbances of the solution from the Tablet and the Standard solution, respectively; and 406.37 and 397.37 are the molecular weights of codeine phosphate hemihydrate and anhydrous codeine phosphate, respectively.

**Limit of morphine**—A 1-mL portion of the filtrate from *Identification test B* meets the requirements of the test for *Limit of morphine* under *Codeine Phosphate*.

**Assay**—Weigh and finely powder not fewer than 20 Tablets. Accurately weigh a portion of the powder, equivalent to about 150 mg of codeine phosphate, and transfer to a 100-mL volumetric flask. Add 20 mL of 0.5 N sulfuric acid, and shake the mixture occasionally during 2 hours. Add water to volume, mix, and filter through a filtering crucible. Transfer to a separator an accurately measured portion of the filtrate, equivalent to not less than 75 mg of codeine phosphate, render the solution alkaline with 6 N ammonium hydroxide, and completely extract the alkaloid with successive 15-mL portions of chloroform. Evaporate the combined chloroform solution on a steam bath nearly to dryness. Dissolve the residue in about 2 mL of methanol, heating, if necessary, add methyl red TS, and titrate with 0.02 N sulfuric acid VS to a faint pink color. Add about 40 mL of freshly boiled, cooled water, and complete the titration with 0.02 N sulfuric acid VS. Each mL of 0.02 N sulfuric acid is equivalent to 8.128 mg of  $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ .

**Codeine Sulfate**

$(\text{C}_{18}\text{H}_{21}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4 \cdot 3\text{H}_2\text{O}$	750.85
$(\text{C}_{18}\text{H}_{21}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4$	696.82
Morphinan-6-ol, 7,8-didehydro-4,5-epoxy-3-methoxy-17-methyl-, (5 $\alpha$ ,6 $\alpha$ )-, sulfate (2:1) (salt), trihydrate; 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -ol sulfate (2:1) (salt) trihydrate [6854-40-6].	
Anhydrous [1420-53-7].	

**DEFINITION**

Codeine Sulfate, dried at 105° for 3 h, contains NLT 98.0% and NMT 102.0% of  $(\text{C}_{18}\text{H}_{21}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4$ .



**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B. ULTRAVIOLET ABSORPTION (197U)**  
Sample solution: 100 µg/mL in water  
Acceptance criteria: Absorptivities at 284 nm do not differ by more than 3.0%, calculated on the dried basis.
- **C. IDENTIFICATION TESTS—GENERAL, Sulfate (191):** Meets the requirements

**ASSAY**• **PROCEDURE**

**Buffer:** Dissolve 4.0 g of potassium phosphate monobasic in 2000 mL of water, and adjust with phosphoric acid to a pH of 3.0 ± 0.1.

**Solution A:** 5 mM sodium heptane sulfonate in methanol and *Buffer* (3:7). [NOTE—Dissolve 1.0 g sodium heptane sulfonate for each L of *Mobile phase* produced, and filter.]

**Solution B:** 5 mM sodium heptane sulfonate in methanol and *Buffer* (11:9). [NOTE—Dissolve 1.0 g sodium heptane sulfonate for each L of *Mobile phase* produced, and filter.]

**Diluent:** *Solution A*

**Mobile phase:** See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
16	0	100
16.5	100	0
24	100	0

**Standard solution:** 1 mg/mL of USP Codeine Sulfate RS in *Diluent*

**Sample solution:** 1 mg/mL of Codeine Sulfate in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 40°

**Flow rate:** 1.0 mL/min

**Injection size:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of codeine sulfate

$[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4]$  in the portion of Codeine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak area of codeine sulfate from the *Sample solution*

$r_S$  = peak area of codeine sulfate from the *Standard solution*

$C_S$  = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Codeine Sulfate in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

**IMPURITIES****Inorganic Impurities**

- **RESIDUE ON IGNITION (281):** NMT 0.1%

**Organic Impurities**• **PROCEDURE 1**

**Buffer, Solution A, Solution B, Diluent, Mobile phase, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard stock solution:** 1 mg/mL of USP Codeine Sulfate RS in *Diluent*

**Standard solution:** 0.01 mg/mL of USP Codeine Sulfate RS in *Diluent* from *Standard stock solution*

**Sensitivity solution:** 0.5 µg/mL of USP Codeine Sulfate RS in *Diluent* from *Standard stock solution*

**Sample solution:** 1 mg/mL of Codeine Sulfate in *Diluent*

**System suitability**

**Samples:** *Standard solution* and *Sensitivity solution*

**Suitability requirements**

**Tailing factor:** NLT 0.5 and NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Codeine Sulfate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each individual impurity from the *Sample solution*

$r_S$  = peak response of codeine sulfate from the *Standard solution*

$C_S$  = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Codeine Sulfate in the *Sample solution* (mg/mL)

$F$  = relative response factor of the related compounds (see *Impurity Table 1*)

**Acceptance criteria**

**Individual impurities:** See *Impurity Table 1*.

**Total impurities:** NMT 1.5%.

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
10-Hydroxy-codeine <sup>a</sup>	0.81	1.35	0.15
Codeine-N-oxide <sup>b</sup>	0.90	1.0	0.15
Codeine sulfate	1.00	—	—
Norcodeine <sup>c</sup>	1.09	1.0	0.15
Codeinone <sup>d</sup>	1.16	1.0	0.15
Codeine methyl ether <sup>e</sup>	1.34	1.0	1.0
Individual unspecified impurities	—	—	0.10

<sup>a</sup> 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α,10-diol.

<sup>b</sup> 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol N-oxide.

<sup>c</sup> 7,8-Didehydro-4,5α-epoxy-3-methoxymorphinan-6α-ol.

<sup>d</sup> 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-one.

<sup>e</sup> 7,8-Didehydro-4,5α-epoxy-3,6α-dimethoxy-17-methylmorphinan.

• **PROCEDURE 2: LIMIT OF MORPHINE**

**Analysis:** Dissolve 50 mg of potassium ferricyanide in 10 mL of water, and add 1 drop of ferric chloride TS and 1 mL of a 10 mg/mL solution of Codeine Sulfate.



**Acceptance criteria:** No blue color is produced immediately.

#### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation (781S)**  
Sample solution: 20 mg/mL, in water  
Acceptance criteria:  $-112.5^{\circ}$  to  $-115.0^{\circ}$
- **ACIDITY**  
Analysis: Dissolve 500 mg in 15 mL of water, add 1 drop of methyl red TS, and titrate with 0.020 N sodium hydroxide.  
Acceptance criteria: NMT 0.30 mL is required for neutralization.
- **WATER DETERMINATION, Method III (921)**: Dry 500 mg at  $105^{\circ}$  for 3 h: it loses between 6.0% and 7.5% of its weight.
- **READILY CARBONIZABLE SUBSTANCES TEST (271)**  
Sample solution: Dissolve 10 mg in 5 mL of sulfuric acid.  
Acceptance criteria: The solution has no more color than *Matching Fluid S*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Codeine Sulfate RS

### Codeine Sulfate Oral Solution

#### DEFINITION

Codeine Sulfate Oral Solution contains NLT 93.0% and NMT 105.0% of the labeled amount of codeine sulfate  $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4]$ .

#### IDENTIFICATION

- **A**. The retention time of the codeine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B. THIN-LAYER CHROMATOGRAPHY**  
Diluent: Methanol and water (25:75)  
Standard solution: 1 mg/mL of USP Codeine Sulfate RS in Diluent  
Sample solution: 1 mg/mL of codeine sulfate in Diluent  
Chromatographic system  
Mode: TLC  
Adsorbent: 0.25-mm layer of chromatographic silica gel mixture  
Application volume: 10  $\mu$ L  
Developing solvent system: Methanol, ethyl acetate, water, and ammonium hydroxide (135: 85: 1: 0.5)

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Develop the plate until the solvent front has moved about three-fourths of the length of the plate, remove it, mark the solvent front, and allow the solvent to evaporate. Place the plate in an iodine chamber, and visualize it for NLT 5 min.

**Acceptance criteria:** The principal spot from the *Sample solution* corresponds in  $R_f$  value to that from the *Standard solution*.

#### ASSAY

##### PROCEDURE

[NOTE—Solutions are stable for 4 days at room temperature when stored in amber glassware.]

**Buffer:** 0.02 M ammonium acetate. Dissolve 1.54 g of ammonium acetate in 1 L of water, and adjust with glacial acetic acid to a pH of  $4.2 \pm 0.1$ .

**Solution A:** Dissolve 1.71 g of sodium decanesulfonic acid in 1 L of methanol and *Buffer* mixture (33:67). Pass through a filter of 0.45- $\mu$ m pore size.

**Solution B:** Dissolve 1.71 g of sodium decanesulfonic acid in 1 L of methanol and *Buffer* mixture (63:37). Pass through a filter of 0.45- $\mu$ m pore size.

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
3.3	60	40
20	0	100
21	0	100
22	100	0
28	100	0

**Diluent:** Methanol and water (25:75)

**Standard solution:** 1.2 mg/mL of USP Codeine Sulfate RS in *Diluent*. Prepare by adding 70% of the flask volume of *Diluent*, and sonicate to dissolve. Dilute with *Diluent* to volume.

**Sample solution:** 1.2 mg/mL of codeine sulfate in *Diluent*, prepared by adding 70% of the flask volume of *Diluent*, then swirling, and letting it sit for 10 min. Dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  15-cm; 3.5- $\mu$ m packing L1

**Column temperature:**  $40 \pm 2^{\circ}$

**Flow rate:** 1.2 mL/min

**Injection volume:** 30  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of codeine sulfate  $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4]$  in each mL of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of codeine sulfate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–105.0%

#### IMPURITIES

##### ORGANIC IMPURITIES

**Buffer, Solution A, Solution B, Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**Sensitivity solution:** 0.6  $\mu$ g/mL of USP Codeine Sulfate RS in *Diluent* from the *Standard solution*

#### System suitability

**Samples:** *Standard solution* and *Sensitivity solution*

#### Suitability requirements

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each individual impurity in the portion of Oral Solution taken:

$$(r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = response of each individual impurity from the *Sample solution*  
 $r_S$  = response of codeine sulfate in the *Standard solution*  
 $C_S$  = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of codeine sulfate in the *Sample solution* (mg/mL)

**Acceptance criteria**

**Individual impurities:** See Table 2. Disregard any peak less than 0.05%.

**Table 2**

Related Compound	Relative Retention Time	Acceptance Criteria, NMT (%)
Codeine-N-oxide <sup>a</sup>	0.65	0.15
Codeine	1.00	—
Codeinone <sup>b</sup>	1.16	0.15
Individual unspecified degradant	—	0.15
Total impurities	—	0.5

<sup>a</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -ol N-oxide.

<sup>b</sup> 7,8-Didehydro-4,5 $\alpha$ -epoxy-3-methoxy-17-methylmorphinan-6 $\alpha$ -one.

**SPECIFIC TESTS**

- MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count does not exceed 10<sup>2</sup> cfu/mL. The total yeasts and molds count does not exceed 20 cfu/mL. It meets the requirements of the test for absence of *Escherichia coli*.
- pH** (791): 2.8–3.8

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Store at controlled room temperature in a well-sealed container.
- USP REFERENCE STANDARDS** (11)  
USP Codeine Sulfate RS

**Codeine Sulfate Tablets****DEFINITION**

Codeine Sulfate Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of codeine sulfate trihydrate  $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O]$ .

**IDENTIFICATION**

- A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- B.**  
**Standard solution and Sample solution:** Proceed as directed in the Assay.  
**Analysis:** Inject 2  $\mu$ L each of the *Standard solution* and the *Sample solution* using the *Chromatographic system* in the Assay.  
**Acceptance criteria:** The spectrum of the codeine peak of the *Sample solution* exhibits maxima and minima at the same wavelengths as those of the corresponding peaks of the *Standard solution*, as obtained in the Assay.

**ASSAY****PROCEDURE**

- Solution A:** Acetonitrile and 0.1% ammonium hydroxide (1.0 mL of concentrated ammonium hydroxide and 1000 mL of water) (1:19)  
**Solution B:** Acetonitrile and 0.1% ammonium hydroxide (9:11)

**Mobile phase:** See Table 1.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
40	0	100
41	100	0
50	100	0

[NOTE—The *Standard solution* and *Sample solution*, for the degradation products, are stable for 4 days when stored at room temperature in amber vials.]

**Diluent:** 0.5% phosphoric acid (5 mL of concentrated phosphoric acid and 1000 mL of water)

**Standard solution:** 1.2 mg/mL of USP Codeine Sulfate RS in *Diluent*

**Sample solution:** Nominally 1.2 mg/mL of codeine sulfate trihydrate in *Diluent*. Dissolve 20 Tablets in 80% of the flask volume of *Diluent* and sonicate for 15–30 min with occasional swirling before diluting with *Diluent* to volume.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 282 nm. For *Identification test B*, use a diode-array detector in the range of 210–400 nm.

**Column:** 4.6-mm  $\times$  15-cm; 3- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.2 mL/min

**Injection volume:** 40  $\mu$ L

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of codeine sulfate trihydrate  $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O]$  in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

$r_U$  = peak area of codeine sulfate from the *Sample solution*

$r_S$  = peak area of codeine sulfate from the *Standard solution*

$C_S$  = concentration of USP Codeine Sulfate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of codeine sulfate trihydrate in the *Sample solution* (mg/mL)

$M_{r1}$  = molecular weight of codeine sulfate trihydrate, 750.85

$M_{r2}$  = molecular weight of codeine sulfate, anhydrous, 696.81

**Acceptance criteria:** 93.0%–107.0%

**PERFORMANCE TESTS****DISSOLUTION** (711)

**Medium:** Water; 500 mL

**Apparatus 2:** 25 rpm

**Time:** 45 min

**Detector:** UV maxima at about 284 nm

**Cell:** 1 cm

**Blank:** *Medium*

**Standard solution:** USP Codeine Sulfate RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.8- $\mu$ m pore size.

**Tolerances:** NLT 75% (Q) of the labeled amount of codeine sulfate trihydrate  $[(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 3H_2O]$  is dissolved.

- UNIFORMITY OF DOSAGE UNITS** (905): Meet the requirements



**IMPURITIES**• **ORGANIC IMPURITIES**

**Solution A, Solution B, Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system:** Proceed as directed in the Assay.

**Sensitivity solution:** 0.6 µg/mL of USP Codeine Sulfate RS from the Standard solution in Diluent

**System suitability**

**Samples:** Standard solution and Sensitivity solution  
**Suitability requirements**

**Tailing factor:** NMT 2.0, Standard solution

**Relative standard deviation:** NMT 2.0%, Standard solution

**Signal-to-noise ratio:** NLT 10, Sensitivity solution

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of each individual impurity in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of each individual impurity from the Sample solution

$r_s$  = peak response of codeine sulfate from the Standard solution

$C_s$  = concentration of USP Codeine Sulfate RS in the Standard solution (mg/mL)

$C_u$  = nominal concentration of codeine sulfate trihydrate in the Sample solution (mg/mL)

$F$  = relative response factor (see Table 2)

**Acceptance criteria:** See Table 2. Disregard any impurity peak less than 0.05%.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Codeine-N-oxide <sup>a</sup>	0.39	1.25	0.2
Codeine sulfate	1.00	—	—
Codeinone <sup>b</sup>	1.10	1.0	0.3
Individual unspecified degradant	—	1.0	0.2
Total impurities	—	—	0.5

<sup>a</sup> 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol N-oxide.

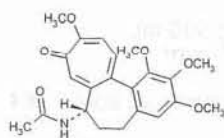
<sup>b</sup> 7,8-Didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-one.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature. Protect from moisture and light.

• **USP REFERENCE STANDARDS (11)**

USP Codeine Sulfate RS

**Colchicine**

$C_{22}H_{25}NO_6$  399.44

Acetamide, N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl)-, (S)-.

Colchicine [64-86-8].

» Colchicine is an alkaloid contained in various species of *Colchicum* and in other genera. It contains not less than 94.0 percent and not more than 101.0 percent of  $C_{22}H_{25}NO_6$ , calculated on the anhydrous, solvent-free basis.

**Caution—Colchicine is extremely poisonous.**

**Packaging and storage—**Preserve in tight, light-resistant containers.

**USP Reference standards (11)—**

USP Colchicine RS

**Identification, Infrared Absorption (197K)—**[NOTE—Disregard any peak occurring at  $1735\text{ cm}^{-1}$ .]

**Specific rotation (781S):** between  $-240^\circ$  and  $-250^\circ$ , calculated on the anhydrous, solvent-free basis.

**Test solution:** 10 mg per mL, in alcohol.

**Water Determination, Method I (921):** not more than 2.0%.

**Limit of colchicine—**To 5 mL of a solution (1 in 100) add 2 drops of ferric chloride TS: no definite green color is produced.

**Limit of ethyl acetate—**

**Internal standard solution—**Dilute 0.5 mL of *n*-propyl alcohol with water to 100.0 mL.

**Standard solution—**Pipet 1 mL of ethyl acetate and 0.5 mL of *n*-propyl alcohol into a 1000-mL volumetric flask, add water to volume, and mix. Each mL of Standard solution contains 0.90 mg of ethyl acetate.

**Test solution—**Place about 250 mg of Colchicine, accurately weighed, in a 10-mL volumetric flask, dissolve in about 8 mL of water, and add 1.0 mL of Internal standard solution. Add water to volume, and mix.

**Procedure—**Determine appropriate sensitivity settings on a gas chromatograph (see Chromatography (621)) fitted with a 4-mm × 1.5-m column packed with 20% (w/v) phase G14 on support S1, maintaining the column temperature at  $75^\circ$ , using nitrogen as the carrier gas, and using a flame-ionization detector. Inject the Standard solution and the Test solution, determine the peak height for ethyl acetate relative to the peak height for *n*-propyl alcohol, and calculate the percentage, by weight, of ethyl acetate in the portion of Colchicine taken: not more than 8.0% is found.

**Chromatographic purity—**The sum of the responses of any peaks other than that due to colchicine, eluting within 1.5 times the retention time for colchicine, is not more than 5.0% of the sum of all responses, obtained as directed in the Assay.

**Residual solvents (467):** meets the requirements, except that the limit of chloroform is 100 ppm.

**Assay—**[NOTE—Perform all dilutions in low-actinic glassware.]

**Mobile phase—**Dilute 45 mL of 0.5 M monobasic potassium phosphate with water to 450 mL. Add about 530 mL of methanol, cool to room temperature, and add methanol to bring the volume to 1000 mL. Adjust with 0.5 M phosphoric acid to a pH of  $5.5 \pm 0.05$ , and pass through a 0.45-µm membrane filter.

**Standard preparation—**Dissolve an accurately weighed quantity of USP Colchicine RS in a mixture of methanol and water (1:1), and dilute quantitatively and stepwise with the same mixture to obtain a solution having a known concentration of about 6 µg per mL. This solution is stable for 4 months when stored tightly stoppered and in the dark.

**Assay preparation—**[NOTE—Prepare immediately before use.] Transfer about 60 mg of Colchicine, accurately weighed, to a 500-mL volumetric flask, dissolve in a mixture of methanol and water (1:1), dilute with the same mixture to volume, and mix. Pipet 5 mL of this solution into a 100-mL volumetric flask, dilute with the same mixture to volume, and mix.



**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 4500 theoretical plates, the retention time for colchicine is between 5.5 and 9.5 minutes, and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for all peaks recorded during 1.5 times the retention time for colchicine. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub> in the Colchicine taken by the formula:

$$10C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of the *Standard preparation*; and  $r_U$  and  $r_S$  are the colchicine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Colchicine Injection

» Colchicine Injection is a sterile solution of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub> in Water for Injection, prepared from Colchicine with the aid of Sodium Hydroxide. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub>.

**Caution**—Colchicine is extremely poisonous.

**Packaging and storage**—Preserve in single-dose containers, preferably of Type I glass, protected from light.

**USP Reference standards** (11)—

USP Colchicine RS

USP Endotoxin RS

**Identification**—

**A:** Transfer a volume of Injection, equivalent to about 2 mg of colchicine, to a separator. Add 5 mL of water, and extract with 15 mL of chloroform. Evaporate the chloroform extract, using mild heat, to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Colchicine RS.

**B:** The UV absorption spectrum of the Injection, diluted with water to a concentration of about 10 µg of colchicine per mL, exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Colchicine RS, concomitantly measured.

**Bacterial Endotoxins Test** (85)—It contains not more than 166.7 USP Endotoxin Units per mg of colchicine.

**pH** (791): between 6.0 and 7.2, in a solution of Injection containing 1.0 mg of potassium chloride in each mL.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—[NOTE—Perform all dilutions in low-actinic glassware.]

**Mobile phase, Standard preparation, and Chromatographic system**—Prepare as directed in the *Assay under Colchicine*.

**Assay preparation**—[NOTE—Prepare immediately before use.] Transfer an accurately measured volume, V mL, of Injection, equivalent to about 1 mg of colchicine, to a 50-mL volumetric flask, dilute with a mixture of methanol and water (1:1) to volume, and mix. Pipet 30 mL of this solution

into a 100-mL volumetric flask, dilute with the same mixture to volume, and mix.

**Procedure**—Proceed as directed for *Procedure* in the *Assay under Colchicine*, and measure the responses for the colchicine peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub> in each mL of the Injection taken by the formula:

$$(C / 6V)(r_U / r_S)$$

in which C is the concentration, in µg per mL, of the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Colchicine Tablets

» Colchicine Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub>.

**Packaging and storage**—Preserve in well-closed, light-resistant containers.

**USP Reference standards** (11)—

USP Colchicine RS

**Identification**—Weigh a portion of ground Tablets, equivalent to about 20 mg of colchicine, triturate with 20 mL of water, allow the solids to settle, and filter the supernatant into a separator. Extract with 30 mL of chloroform. Evaporate the chloroform extract, using mild heat, to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue so obtained exhibits maxima only at the same wavelengths as that of a similar preparation of USP Colchicine RS.

**Dissolution, Procedure for a Pooled Sample** (711)—[NOTE—Conduct this procedure without delay, under subdued light, and using low-actinic glassware.]

**Medium:** water; 500 mL.

**Apparatus 1:** 100 rpm.

**Time:** 30 minutes.

**Procedure**—Determine the amount of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub> dissolved, employing the procedure set forth in the *Assay under Colchicine*, making any necessary modifications.

**Tolerances**—Not less than 75% (Q) of the labeled amount of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub> is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—[NOTE—Perform all dilutions in low-actinic glassware.]

**Mobile phase, Standard preparation, and Chromatographic system**—Prepare as directed in the *Assay under Colchicine*.

**Assay preparation**—[NOTE—Prepare immediately before use.] Weigh and finely powder not fewer than 20 Tablets. Transfer an accurately weighed portion of the powder, equivalent to about 0.6 mg of colchicine, to a 100-mL volumetric flask, add about 50 mL of a mixture of methanol and water (1:1), and shake by mechanical means for 15 minutes, rinsing down the walls of the flask at about 8 minutes. Dilute with the same mixture to volume, and pass through a 0.45-µm membrane filter.

**Procedure**—Proceed as directed for *Procedure* in the *Assay under Colchicine*, and measure the responses for the colchicine peaks. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>25</sub>NO<sub>6</sub> in the portion of Tablets taken by the formula:

$$0.1C(r_U / r_S)$$

in which C is the concentration, in µg per mL, of the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses ob-



tained from the *Assay preparation* and the *Standard preparation*, respectively.

## Colestipol Hydrochloride

» Colestipol Hydrochloride is an insoluble, high molecular weight basic anion-exchange copolymer of diethylenetriamine and 1-chloro-2,3-epoxypropane with approximately one out of five amino nitrogens protonated. Each g binds not less than 1.1 mEq and not more than 1.6 mEq of sodium cholate, calculated as cholate binding capacity.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Colestipol Hydrochloride RS

**Identification**—*Infrared Absorption* (197K)—Prepare the test specimen and the Standard specimen by mixing about 3 to 4 mg of Colestipol Hydrochloride and of USP Colestipol Hydrochloride RS, respectively, with about 150 mg of potassium bromide.

**pH** (791)—Prepare a 10% (w/w) suspension of it in deionized water in a clean vial. Insert the stopper, shake at approximately 10-minute intervals for 1 hour, and centrifuge. Transfer a portion of the clear supernatant to a suitable container, and record the pH as soon as the reading has stabilized: the pH is between 6.0 and 7.5.

**Loss on drying** (731)—Dry it in vacuum at a pressure of about 5 mm of mercury at 75° for 16 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.3%.

**Delete the following:**

• **Heavy metals**, Method II (231): not more than 0.002%.

• (Official 1-Jan-2018)

**Content of chloride**—

**Test preparation**—Using about 20 mg of Colestipol Hydrochloride, accurately weighed, proceed as directed under *Oxygen Flask Combustion* (471), 10 mL of 0.05 N sodium hydroxide being used as the absorbing liquid. Do not allow the paper specimen wrapper to come in contact with the liquid, and ignite the paper with an IR igniter. After combustion is complete, shake the flask vigorously, and allow to stand, with frequent shaking, for about 40 minutes or until no cloudiness is present. Transfer the solution to a 50-mL beaker. Wash the flask with two 5-mL portions of water and two 10-mL portions of alcohol, adding each washing to the beaker, and add 0.2 mL of nitric acid.

**Reagent blank preparation**—Using a paper specimen wrapper, complete the combustion, and allow the mixture to stand for about 40 minutes or until no cloudiness is present, as directed under *Test preparation*. Transfer the solution so obtained to a 50-mL beaker. Wash the combustion flask with two 5-mL portions of water and two 10-mL portions of alcohol, adding the washings to the beaker, and add 0.2 mL of nitric acid.

**Procedure**—Titrate the *Test preparation* and the *Reagent blank preparation* with 0.05 N silver nitrate VS, determining the endpoint potentiometrically, using a silver-silver chloride electrode and a glass reference electrode (see *Titrimetry*

(541)). Determine the volume of 0.05 N silver nitrate VS consumed by the test specimen taken by the formula:

$$V - V_B$$

in which  $V$  and  $V_B$  are the volumes, in mL, of titrant used for the *Test preparation* and the *Reagent blank preparation*, respectively. Each mL of 0.05 N silver nitrate is equivalent to 1.773 mg of Cl: the chloride content is between 6.5% and 9.0%, calculated on the dried basis.

**Water absorption**—Transfer about 5 g of Colestipol Hydrochloride, accurately weighed, to a dry, 100-mL plastic container, and add about 80 g of water, accurately weighed. Cover the container, and allow the suspension to equilibrate for 72 hours. With the aid of vacuum, filter the slurry transferred to a medium-porosity, fritted-glass funnel, and collect the filtrate in a tared plastic container. Disconnect the vacuum 2 minutes after the collection of the last portion of the filtrate. Weigh the container and the filtrate, and determine the weight, in g, of the filtrate. Determine the amount of water absorbed by subtracting the weight of the filtrate from the weight of water taken for the test, and divide the weight, in g, of the absorbed water by the weight, in g, of Colestipol Hydrochloride taken: each g absorbs between 3.3 g and 5.3 g of water.

**Cholate binding capacity**—

**Cholate solution**—Dissolve accurately weighed quantities of sodium cholate and sodium chloride in water, and quantitatively dilute with water to obtain a solution having known concentrations of 10.0 mg of sodium cholate per mL and 9.0 mg of sodium chloride per mL. Adjust the solution by the dropwise addition of hydrochloric acid to a pH of 6.4  $\pm$  0.1.

**Test preparation**—Transfer 1.0  $\pm$  0.01 g of Colestipol Hydrochloride to a glass-stoppered, 125-mL conical flask. Add 100.0 mL of *Cholate solution*, insert the stopper securely in the flask, shake vigorously for 90 minutes with the flask positioned horizontally on a platform shaker, remove the flask from the shaker, and allow the solids to settle for 5 minutes.

**Procedure**—Transfer 20.0 mL of supernatant from the *Test preparation* to a 40-mL beaker, transfer 20.0 mL of *Cholate solution* to a second 40-mL beaker, and adjust both solutions by the dropwise addition of 1 N sodium hydroxide to a pH of 10.5  $\pm$  0.5. Titrate both solutions with 0.1 N hydrochloric acid VS, determining the endpoints potentiometrically, and measure the titrant volume corresponding to the difference between the midpoints of the two inflections in the titration curves obtained for each solution (see *Titrimetry* (541)). Determine the volume of titrant equivalent to the bound cholate by subtracting the volume of 0.1 N hydrochloric acid VS used in titrating the *Test preparation* from that used in titrating the *Cholate solution*. Calculate the *Cholate binding capacity*, in mEq per g, taken by the formula:

$$SVN / W$$

in which  $V$  is the volume, in mL, of titrant equivalent to the bound cholate;  $N$  is the normality of the 0.1 N hydrochloric acid VS; and  $W$  is the weight, in g, of Colestipol Hydrochloride taken for the *Test preparation*. The *Cholate binding capacity* is between 1.1 mEq per g and 1.6 mEq per g.

**Water-soluble substances**—Transfer 5.0 g of Colestipol Hydrochloride, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 80.0 mL of water, insert the stopper in the flask, and mount the flask in a water-bath shaker maintained at 37  $\pm$  1°. Operate the shaker for 72 hours, remove the flask from the shaker, and filter the contents twice—first through a premoistened 0.45- $\mu$ m nylon membrane filter and then through a 0.45- $\mu$ m PVDF filter, collecting the filtrate in a tared 125-mL conical flask. Rinse any residual test material in the flask with two 5-mL portions of water, pass the washings through the filters, and combine the filtrates from the washings with the filtrate from the test mixture. Evaporate the filtrate to dryness, filtered air or ni-



trogen being used, if necessary, to aid in the evaporation. Dry the residue in a vacuum oven maintained at 75° for 1 hour, allow to cool in a desiccator, and weigh: not more than 0.5% of water-soluble substances is found in the portion of Colestipol Hydrochloride taken.

#### **Colestipol exchange capacity—**

**Resin base preparation—**Combine not less than 2 g of Colestipol Hydrochloride and 100 mL of 1 N sodium hydroxide in a 125-mL conical flask, insert a stopper in the flask, secure the flask on a platform shaker, and shake the mixture for 3 to 4 hours. Filter the suspension through a coarse-porosity, fritted-glass funnel, and wash the resin with 500 mL of water. Transfer the resin to a 1000-mL beaker, add 200 mL of water, allow to stand for 10 minutes, filter the suspension, and check the pH of the filtrate. Repeat the washing procedure with 200-mL portions of water until the pH of the filtrate is below 8 (as much as 5000 mL of water may be required). Dry the colestipol base resin so obtained and the funnel at a pressure of about 5 mm of mercury at 60° for 16 hours. Break up any aggregates, and store the *Resin base preparation* in a desiccator.

**Procedure—**Transfer about 1.0 g of the *Resin base preparation* to a 125-mL conical flask, add 100.0 mL of 0.20 N hydrochloric acid, insert a stopper in the flask, and shake the mixture by mechanical means for 2.5 hours. Filter a portion of the suspension through a pledget of glass wool, and transfer 8.0 mL of the filtrate (test preparation) to a 25-mL beaker. Transfer 5.0 mL of the same 0.20 N hydrochloric acid that was used to equilibrate the resin to a second 25-mL beaker, and add 5.0 mL of water. Titrate both solutions with 0.2 N sodium hydroxide VS, determining the endpoints potentiometrically (see *Titrimetry* (541)), and calculate the *Colestipol exchange capacity*, in mEq per g, taken by the formula:

$$(100N / W)[(V_b / 5) - (V_a / 8)]$$

in which *N* is the normality of the sodium hydroxide VS; *W* is the weight, in g, of the *Resin base preparation* taken; *V<sub>b</sub>* is the volume, in mL, of titrant used to neutralize the 5.0-mL aliquot of 0.20 N hydrochloric acid; and *V<sub>a</sub>* is the volume, in mL, of titrant used to neutralize the residual acid in the test preparation. Each g exchanges not less than 9.0 mEq and not more than 11.0 mEq of sodium hydroxide, calculated as colestipol exchange capacity.

### **Colestipol Hydrochloride for Oral Suspension**

» Colestipol Hydrochloride for Oral Suspension is a mixture of Colestipol Hydrochloride with a suitable flow-promoting agent. Each g binds not less than 1.1 mEq and not more than 1.6 mEq of sodium cholate, calculated as the cholate binding capacity.

**Packaging and storage—**Preserve in tight, single-dose or multiple-dose containers.

#### **USP Reference standards (11)—**

USP Colestipol Hydrochloride RS

**Identification, Infrared Absorption (197K)—**Prepare the test specimen and the Standard specimen by mixing about 5 mg of Colestipol Hydrochloride for Oral Suspension and 5 mg of USP Colestipol Hydrochloride RS, respectively, with about 100 to 125 mg of potassium bromide.

**Minimum fill (755):** meets the requirements for powders.

**Water-soluble substances—**Transfer 5.0 g of Colestipol Hydrochloride for Oral Suspension, accurately weighed, to a glass-stoppered, 125-mL conical flask, add 80.0 mL of water, insert the stopper in the flask, and mount the flask in a water-bath shaker maintained at 37 ± 1°. Operate the shaker for 72 hours, remove the flask from the shaker, and filter the contents twice—first through a premoistened 0.45-μm nylon membrane filter and then through a 0.45-μm PVDF filter, collecting the filtrate in a tared 100-mL fused quartz crucible. Rinse any residual test material in the flask with two 5-mL portions of water, pass the washings through the filters, and combine the filtrates from the washings with the filtrate from the test mixture. Evaporate the filtrate to dryness, filtered air or nitrogen being used, if necessary, to aid in the evaporation. Dry the residue in a vacuum oven maintained at 75° for 1 hour, allow to cool in a desiccator, and weigh. Calculate the initial percentage of water-soluble substances in the portion of Colestipol Hydrochloride for Oral Suspension taken. Again heat the residue in a muffle furnace maintained at 800 ± 25° for 4 hours, allow to cool in a desiccator, and weigh. Calculate the percentage of inert ingredients present. Calculate the actual percentage of water-soluble substances in the portion of Colestipol Hydrochloride for Oral Suspension taken by subtracting the percentage of inert ingredients from the initial percentage of water-soluble substances found. Not more than 0.5% of water-soluble substances is found.

**Other requirements:** meets the requirements of the tests for *Cholate binding capacity* and *pH* under *Colestipol Hydrochloride*.

### **Colestipol Hydrochloride Tablets**

» Colestipol Hydrochloride Tablets contain Colestipol Hydrochloride. Each Tablet binds not less than 1.1 mEq and not more than 1.6 mEq of sodium cholate per g of the labeled amount of colestipol hydrochloride, calculated as cholate binding capacity.

**Packaging and storage—**Preserve in tight containers, and store at controlled room temperature.

#### **USP Reference standards (11)—**

USP Colestipol Hydrochloride RS

#### **Identification, Infrared Absorption (197K)—**

**Test specimen—**Completely remove the coating film from a Tablet with a suitable implement, and grind the contents into fine powder. To about 30 to 40 mg of the powder, add about 15 mL of methanol, shake vigorously for 3 minutes, then sonicate for 10 minutes, and shake for another 3 minutes. Pass through a suitable paper filter, wash the residue 3 times, each time with 10 mL of methanol. [NOTE—A qualitative paper filter, with a coarse porosity and a particle retention 20–25 μm, is suitable.] Dry the residue at 60° in vacuum for 2 hours. Mix about 4 mg of the dried sample with about 150 mg of potassium bromide.

**Standard specimen—**Mix about 3 to 4 mg of USP Colestipol Hydrochloride RS with about 150 mg of potassium bromide.

#### **Uniformity of dosage units—**

**Sodium chloride solution, Cholate solution, 0.09 M Buffer solution pH 2.5, Mobile phase, Standard preparation, and Chromatographic system—**Proceed as directed in the test for *Cholate binding capacity*.

**Test solution—**Transfer 1 Tablet to a 100-mL volumetric flask, dilute with *Cholate solution* to volume, and stir for 120 minutes. Let the sample settle down for at least 10 min-



utes, and filter a portion using a 0.45- $\mu$ m PVDF filter, discarding the first 5 mL of the filtrate.

**Procedure**—Proceed as directed in the test for *Cholate binding capacity*, except to inject the *Test solution* instead of the *Test preparation*.

Select not fewer than 30 Tablets. Test 10 Tablets individually as directed above. The requirements are met if the cholate binding capacity in each of the 10 Tablets lies within the range of 1.15 to 1.55 mEq per g of the labeled amount of colestipol hydrochloride, and the relative standard deviation is not more than 6.0%.

If 1 Tablet is outside the range of 1.15 to 1.55 mEq per g and no Tablet is outside the range of 1.01 to 1.69 mEq per g, or if the relative standard deviation is greater than 6.0%, or if both conditions prevail, test 20 additional Tablets. The requirements are met if not more than 1 Tablet of the 30 is outside the range of 1.15 to 1.55 mEq per g and no Tablet is outside the range of 1.01 to 1.69 mEq per g of the labeled amount of colestipol hydrochloride, and the relative standard deviation for 30 Tablets does not exceed 7.8%.

**pH** (791)—Transfer 5 g of ground Tablets to a suitable flask, add 50 mL of deionized water, close the flask with a stopper, and stir for about 30 minutes or until the Tablets completely disintegrate. Centrifuge to obtain a clear supernatant: the pH is between 5.5 and 7.5.

#### Cholate binding capacity—

**0.09 M Buffer solution pH 2.5**—Dissolve 12.5 g of monobasic sodium phosphate in 1000 mL of water, and adjust with phosphoric acid to a pH of  $2.5 \pm 0.05$ .

**Mobile phase**—Prepare a mixture of 0.09 M Buffer solution pH 2.5, acetonitrile, and methanol (50:36:14), mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Sodium chloride solution**—Prepare a solution in water containing 9.0 mg of sodium chloride per mL.

**Cholate solution**—Transfer an accurately weighed quantity of sodium cholate to a suitable volumetric flask, add *Sodium chloride solution* to about 80% of the final volume, sonicate to dissolve, and dilute with *Sodium chloride solution* to volume to obtain a solution having a known concentration of 10.0 mg of sodium cholate per mL on the anhydrous basis. [NOTE—Determine the water content of sodium cholate titrimetrically at the time of use.] Adjust the solution by the dropwise addition of 0.5 N hydrochloric acid to a pH of  $6.45 \pm 0.05$ . [NOTE—Do not allow the pH to go below 6.40 at any time.] Use this solution as soon as possible after preparation.

**Test preparation**—Transfer 10 Tablets to a glass-stoppered 1.5-L flask. Add 1000.0 mL of *Cholate solution*, insert the stopper securely in the flask, and stir for 120 minutes. Filter a portion using a 0.45- $\mu$ m PVDF filter, discarding the first 5 mL of the filtrate.

**Standard preparation**—Dilute a portion of the *Cholate solution* with *Sodium chloride solution*, to obtain a solution having a known concentration of about 4.0 mg of sodium cholate per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L7. The flow rate is about 1.2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 2000 theoretical plates; the tailing is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the concen-

tration of the unbound cholate,  $C_T$ , in the *Test preparation* by the formula:

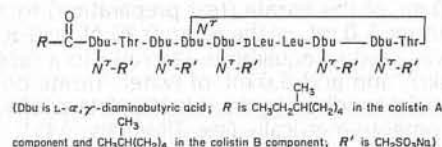
$$C_S \times (r_U / r_S)$$

in which  $C_S$  is the concentration, in mg per mL, of sodium cholate in the *Standard preparation*; and  $r_U$  and  $r_S$  are the cholate peak areas obtained from the *Test preparation* and the *Standard preparation*, respectively. Calculate the cholate binding capacity, in mEq per g of the labeled amount of colestipol hydrochloride, by the formula:

$$(1000 / 430.6)(C_{CH} - C_T) / NL$$

in which 1000 is a conversion coefficient to g; 430.6 is the molecular weight of sodium cholate;  $C_{CH}$  is the concentration, in mg per mL, of sodium cholate in the *Cholate solution*;  $N$  is the number of Tablets taken to prepare the *Test preparation*; and  $L$  is the labeled amount of colestipol hydrochloride, in g per Tablet.

## Colistimethate Sodium



$\text{C}_{58}\text{H}_{105}\text{N}_{16}\text{Na}_5\text{O}_{28}\text{S}_5$  (colistin A component) 1749.82

$\text{C}_{57}\text{H}_{103}\text{N}_{16}\text{Na}_5\text{O}_{28}\text{S}_5$  (colistin B component) 1735.79

Colistimethate sodium.

Pentasodium colistinmethanesulfonate [8068-28-8; 21362-08-3].

» Colistimethate Sodium has a potency equivalent to not less than 390  $\mu$ g of colistin per mg.

#### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

**Labeling**—Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

#### USP Reference standards (11)—

USP Colistimethate Sodium RS

USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

**Identification, Infrared Absorption** (197K).

**pH** (791): between 6.5 and 8.5, in a solution containing 10 mg per mL.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 7.0% of its weight.

#### Delete the following:

• **Heavy metals, Method II** (231): not more than 0.003%.

• (Official 1-Jan-2018)



**Free colistin**—Dissolve 80 mg in 3 mL of water, and add 0.05 mL of silicotungstic acid solution (1 in 10): no immediate precipitate is formed.

**Other requirements**—Where the label states that Colistimethate Sodium is sterile, it meets the requirements for *Sterility* and *Bacterial endotoxins* under *Colistimethate for Injection*. Where the label states that Colistimethate Sodium must be subjected to further processing during the preparation of injectable dosage forms, it meets the requirements for *Bacterial endotoxins* under *Colistimethate for Injection*.

#### Change to read:

#### Assay—

**Assay preparation**—Dissolve a suitable quantity of Colistimethate Sodium, accurately weighed, in 2.0 mL of water, add a sufficient accurately measured volume of *Buffer B.6* (CN 1-May-2017) to obtain a solution having a convenient concentration.

**Procedure**—Proceed as directed for Colistimethate Sodium under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer B.6* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Colistimethate for Injection

» Colistimethate for Injection contains an amount of Colistimethate Sodium equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of colistin.

#### Change to read:

**Packaging and storage**—Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).

#### USP Reference standards (11)—

USP Colistimethate Sodium RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

**Bacterial Endotoxins Test** (85)—It contains not more than 2.0 USP Endotoxin Units per mg of colistin.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

#### Change to read:

**Other requirements**—It responds to the *Identification* test and meets the requirements for *pH*, *Loss on drying*, *Official 1-Jan-2018* and *Free colistin* under *Colistimethate Sodium*. It meets also the requirements for *Uniformity of Dosage Units* (905) and for *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions* and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

#### Change to read:

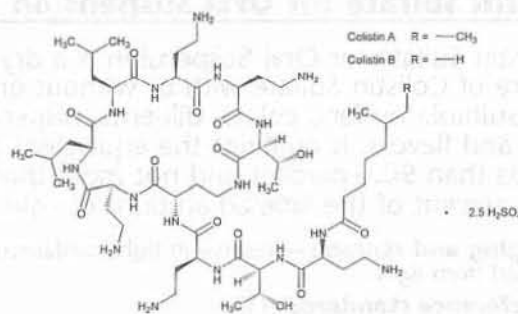
#### Assay—

**Assay preparation 1** (where it is represented as being in a single-dose container)—Constitute Colistimethate for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Withdraw all of the withdrawable contents, using a suitable hypodermic needle and syringe, and quantitatively dilute with *Buffer B.6* (CN 1-May-2017) to obtain a solution having a convenient concentration.

**Assay preparation 2** (where the label states the quantity of colistin equivalent in a given volume of constituted solution)—Constitute Colistimethate for Injection in a volume of water, accurately measured, corresponding to the volume of diluent specified in the labeling. Quantitatively dilute an accurately measured volume of the constituted solution with *Buffer B.6* (CN 1-May-2017) to obtain a solution having a convenient concentration.

**Procedure**—Proceed as directed for Colistimethate Sodium under *Antibiotics—Microbial Assays* (81), using an accurately measured volume of *Assay preparation* diluted quantitatively with *Buffer B.6* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

## Colistin Sulfate



Colistin, sulfate.  
Colistins sulfate [1264-72-8].

» Colistin Sulfate is the sulfate salt of an antibacterial substance produced by the growth of *Bacillus polymyxa* var. *colistinus*. It has a potency equivalent to not less than 500 µg of colistin per mg.

**Packaging and storage**—Preserve in tight containers.

#### USP Reference standards (11)—

USP Colistin Sulfate RS

#### Identification—

**A:** Dissolve about 20 mg in 2 mL of buffer solution, prepared by adjusting 50 mL of 1 M monobasic potassium phosphate with 1 N sodium hydroxide to a pH of 7.0, diluting with water to 100 mL, and mixing. Add 0.2 mL of ninhydrin solution (1 in 200) and boil: a purple color is produced.

**B:** It meets the requirements of the tests for *Sulfate* (191).

**C:** *Liquid Chromatographic Identification Test*—

**Mobile phase**—Prepare a mixture of 0.1 M tribasic sodium phosphate and acetonitrile (77:23), and adjust with phosphoric acid to a pH of 3.0. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).



**Standard solution**—Prepare a solution of USP Colistin Sulfate RS in *Mobile phase* having a concentration of about 6 mg per mL. Protect this solution from light.

**Test solution**—Prepare a solution of Colistin Sulfate in *Mobile phase* having a concentration of about 6 mg per mL. Protect this solution from light.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 212-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1 mL per minute.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard solution* and the *Test solution* into the chromatograph, and record the chromatograms. The chromatogram obtained from the *Test solution* corresponds qualitatively with that obtained from the *Standard solution*, exhibiting a major peak corresponding to colistin A and minor peaks at relative retention times of about 0.55 (colistin B) and 0.8.

**pH** (791): between 4.0 and 7.0, in a solution containing 10 mg per mL.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 7.0% of its weight.

**Assay**—Proceed as directed for Colistin under *Antibiotics—Microbial Assays* (81).

### Colistin Sulfate for Oral Suspension

» Colistin Sulfate for Oral Suspension is a dry mixture of Colistin Sulfate with or without one or more suitable buffers, colors, diluents, dispersants, and flavors. It contains the equivalent of not less than 90.0 percent and not more than 120.0 percent of the labeled amount of colistin.

**Packaging and storage**—Preserve in tight containers, protected from light.

**USP Reference standards** (11)—

USP Colistin Sulfate RS

**Uniformity of dosage units** (905)—

FOR SOLID PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

**Deliverable volume** (698): meets the requirements.

**pH** (791): between 5.0 and 6.0, in the suspension constituted as directed in the labeling.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 hours; it loses not more than 3.0% of its weight.

#### Change to read:

**Assay**—Constitute Colistin Sulfate for Oral Suspension as directed in the labeling. Proceed as directed under *Antibiotics—Microbial Assays* (81) using an accurately measured volume of this suspension, freshly mixed and free from air bubbles, diluted quantitatively with *Buffer B.6* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

### Colistin and Neomycin Sulfates and Hydrocortisone Acetate Otic Suspension

» Colistin and Neomycin Sulfates and Hydrocortisone Acetate Otic Suspension is a sterile suspension containing the equivalent of not less than 90.0 percent and not more than 135.0 percent of the labeled amount of colistin, not less than 90.0 percent and not more than 125.0 percent of the labeled amount of neomycin, and not less than 90.0 percent and not more than 110.0 percent of the labeled amount of hydrocortisone acetate (C<sub>23</sub>H<sub>32</sub>O<sub>6</sub>). It contains one or more suitable buffers, detergents, dispersants, and preservatives.

**NOTE**—Where Colistin and Neomycin Sulfates and Hydrocortisone Acetate Otic Suspension is prescribed, without reference to the quantity of colistin, neomycin, or hydrocortisone acetate contained therein, a product containing 3.0 mg of colistin, 3.3 mg of neomycin, and 10 mg of hydrocortisone acetate per mL shall be dispensed.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Colistin Sulfate RS

USP Neomycin Sulfate RS

USP Hydrocortisone Acetate RS

**Sterility Tests** (71): meets the requirements, 0.25 mL from each container being transferred directly to 90 mL of each medium.

**pH** (791): between 4.8 and 5.2.

#### Change to read:

**Assay for colistin**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using a freshly mixed, accurately measured volume of Otic Suspension diluted quantitatively and stepwise with *Buffer B.6* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

#### Change to read:

**Assay for neomycin**—Proceed as directed under *Antibiotics—Microbial Assays* (81), using a freshly mixed, accurately measured volume of Otic Suspension diluted quantitatively and stepwise with *Buffer B.3* (CN 1-May-2017) to yield a *Test Dilution* having a concentration assumed to be equal to the median dose level of the Standard.

**Assay for hydrocortisone acetate**—

**Reagent blank**—Dilute 200 mL of 22 N sulfuric acid with 100 mL of dehydrated alcohol.

**Phenylhydrazine reagent**—Dissolve 43.33 mg of phenylhydrazine hydrochloride in 100 mL of *Reagent blank*.

**Standard preparation**—Dissolve a suitable quantity of USP Hydrocortisone Acetate RS, accurately weighed, in chloroform, and dilute quantitatively and stepwise with chloroform to obtain a solution having a known concentration of about 10 μg per mL.

**Assay preparation**—Transfer 5.0 mL of freshly mixed Otic Suspension to a 125-mL separator. Extract with three 20-mL portions of chloroform, filtering each chloroform extract



through a pledget of cotton previously saturated with chloroform, collect the filtrates in a 100-mL volumetric flask, dilute with chloroform to volume, and mix. Pipet 10 mL of this solution into a 100-mL volumetric flask, dilute with chloroform to volume, and mix. Pipet 20 mL of this solution into a 100-mL volumetric flask, dilute with chloroform to volume, and mix.

**Procedure**—Pipet 50 mL each of the *Standard* and the *Assay preparation* into separate 125-mL separators, add 2 mL of 0.1 N sodium hydroxide to each separator, shake, and allow the layers to separate. Filter both chloroform layers through glass wool, and collect the filtrates in separate beakers. Pipet two 20-mL portions of each chloroform filtrate into separate 125-mL separators. Add 25.0 mL of *Phenylhydrazine reagent* to one separator each of the filtrates from the *Standard preparation* and the *Assay preparation*, respectively, and add 25.0 mL of *Reagent blank* to the remaining two separators. Shake all four separators well, allow the layers to separate, and discard the chloroform layers. Drain the aqueous layers into separate centrifuge tubes, and centrifuge for 2 minutes. Pipet 10 mL of each solution into separate glass-stoppered test tubes. Place the tubes in a water bath maintained at a temperature of 60° for 30 minutes, then cool the solution to room temperature. Concomitantly determine the absorbances of the solutions at the wavelength of maximum absorbance at about 410 nm, with a suitable spectrophotometer, using water to set the instrument. Calculate the quantity, in mg, of hydrocortisone acetate ( $C_{23}H_{32}O_6$ ) in each mL of the Otic Suspension taken by the formula:

$$C(A_U - A_{UB} / A_S - A_{SB})$$

in which C is the concentration, in µg per mL, of USP Hydrocortisone Acetate RS in the *Standard preparation*;  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation* treated with *Phenylhydrazine reagent*, respectively; and  $A_{UB}$  and  $A_{SB}$  are the absorbances of the solution from the *Assay preparation* and the *Standard preparation* treated with the *Reagent blank*, respectively.

## Collodion

### DEFINITION

Collodion contains NLT 5.0%, by weight, of pyroxylin.

Pyroxylin	40 g
Ether	750 mL
Alcohol	250 mL
To make about	1000 mL

To the *Pyroxylin* in a suitable container add the *Alcohol* and *Ether*, and insert the stopper into the container. Shake the mixture occasionally until the *Pyroxylin* is dissolved.

**[CAUTION]**—Collodion is highly flammable.]

### IDENTIFICATION

- **A.** **Analysis:** Expose a thin layer to air, leaving a transparent, tenacious film.  
**Acceptance criteria:** The film of pyroxylin so obtained burns rapidly with a yellow flame.
- **B.** **Analysis:** Mix with an equal volume of water.  
**Acceptance criteria:** A viscid, stringy mass of pyroxylin is produced.

### ASSAY

#### • PROCEDURE

**Sample:** 10 mL

**Analysis:** Quickly pour the *Sample* into a tared flask, insert the stopper, and weigh the *Assay charge* accurately. Remove the stopper, warm on a steam bath, and add 10 mL of water dropwise, with constant stirring. Evaporate the mixture on a steam bath, and dry the residue at 105° for 4 h.

**Acceptance criteria:** NLT 5.0%, by weight

### OTHER COMPONENTS

#### • ALCOHOL DETERMINATION (611)

**Internal standard solution:** Acetone and 1,2-dichloroethane (20:80) in a glass-stoppered graduated cylinder

**Standard stock solutions:** Transfer 10-, 20-, and 30-mL portions of dehydrated alcohol into separate 100-mL volumetric flasks, dilute with 1,2-dichloroethane to volume, and mix.

**Standard solutions:** Mix 10 mL of each *Standard stock solution* with 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution* in separate glass-stoppered, 50-mL graduated cylinders.

**Sample solution:** To 10 mL of *Collodion* in a glass-stoppered, 50-mL graduated cylinder add 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution*. Mix, and allow the precipitate to settle.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Thermal conductivity

**Column:** 1.8-m × 3.5-mm glass; packing S3

**Temperatures**

**Column:** 150°

**Injection port:** 200°

**Detector:** 250°

**Carrier gas:** Helium

**Flow rate:** 50 mL/min

**Injection volume:** 4 µL

#### Analysis

**Samples:** *Standard solutions* and *Sample solution*

Calculate the relative response factor,  $F$ , for each *Standard solution* taken:

$$F = C_S / R_S$$

$C_S$  = concentration of alcohol in the *Standard solution*, as a percentage (v/v)

$R_S$  = peak response ratio of alcohol to acetone from the respective *Standard solution*

Calculate the percentage of alcohol ( $C_2H_5OH$ ) in the portion of *Collodion* taken:

$$\text{Result} = R_U \times F_a$$

$R_U$  = peak response ratio of alcohol to acetone from the *Sample solution*

$F_a$  = average of the individual  $F$  values

**Acceptance criteria:** 22.0%–26.0%

### SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 0.765–0.775

#### • ACIDITY

**Sample:** 5 mL

**Analysis:** Add the *Sample* to 5 mL of water.

**Acceptance criteria:** The liquid separated from the pyroxylin is not acid to litmus.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight containers, at a temperature not exceeding 30°, remote from fire.
- **LABELING:** The label bears a caution statement to the effect that *Collodion* is highly flammable.



## Flexible Collodion

### DEFINITION

Prepare Flexible Collodion as follows.

Camphor	20 g
Castor Oil	30 g
Collodion, a sufficient quantity to make	1000 g

Weigh the ingredients, successively, into a dry, tared bottle. Insert the stopper in the bottle, and shake the mixture until the *Camphor* is dissolved.

### IDENTIFICATION

- **A.**  
**Analysis:** Expose a thin layer to air, leaving a transparent, tenacious film.  
**Acceptance criteria:** The film exhibits a distinct odor of camphor. The film of pyroxylin so obtained burns rapidly with a yellow flame.
- **B.**  
**Analysis:** Mix with an equal volume of water.  
**Acceptance criteria:** A viscid, stringy mass of pyroxylin is produced.

### OTHER COMPONENTS

#### • ALCOHOL DETERMINATION (611)

**Internal standard solution:** Acetone and 1,2-dichloroethane (20:80) in a glass-stoppered, graduated cylinder  
**Standard stock solutions:** Transfer 10-, 20-, and 30-mL portions of dehydrated alcohol into separate 100-mL volumetric flasks, dilute with 1,2-dichloroethane to volume, and mix.

**Standard solutions:** Mix 10 mL of each *Standard stock solution* with 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution* in separate, glass-stoppered, 50-mL graduated cylinders.

**Sample solution:** To 10 mL of Flexible Collodion in a glass-stoppered, 50-mL graduated cylinder add 15 mL of 1,2-dichloroethane, 10 mL of hexane, and 10.0 mL of *Internal standard solution*. Mix, and allow the precipitate to settle.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Thermal conductivity

**Column:** 1.8-m × 3.5-mm glass; support S3

**Temperatures**

**Column:** 150°

**Injection port:** 200°

**Detector:** 250°

**Carrier gas:** Helium

**Flow rate:** 50 mL/min

**Injection volume:** 4 µL

#### Analysis

**Samples:** *Standard solutions* and *Sample solution*

Calculate the relative response factor, *F*, for each *Standard solution* taken:

$$F = C_S/R_S$$

*C<sub>S</sub>* = concentration of alcohol in the *Standard solution*, as a percentage (v/v)

*R<sub>S</sub>* = peak response ratio of alcohol to acetone from the respective *Standard solution*

Calculate the percentage of alcohol (C<sub>2</sub>H<sub>5</sub>OH) in the portion of Flexible Collodion taken:

$$\text{Result} = R_U \times F_A$$

*R<sub>U</sub>* = peak response ratio of alcohol to acetone from the *Sample solution*

*F<sub>A</sub>* = average of the individual *F* values

**Acceptance criteria:** 21.0%–25.0%

### SPECIFIC TESTS

- **SPECIFIC GRAVITY (841):** 0.770–0.790

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in tight containers, at a temperature not exceeding 30°, remote from fire.
- **LABELING:** The label bears a caution statement to the effect that Flexible Collodion is highly flammable.

## Colloidal Oatmeal

### DEFINITION

Colloidal Oatmeal is the powder resulting from the grinding and further processing of whole oat grain meeting U.S. Standards for Number 1 or Number 2 oats (7 CFR 810.1001).

### IDENTIFICATION

- **A.**  
**Analysis:** Prepare a smooth mixture of 10 g of Colloidal Oatmeal and 100 mL of warm water. Stir for 10 min.  
**Acceptance criteria:** The resulting slurry has a characteristic slippery feel and shows the development of slimy, viscous strands.
- **B.** A water slurry is colored reddish violet to deep blue by iodine TS.

### IMPURITIES

- **ARTICLES OF BOTANICAL ORIGIN, Total Ash (561):** NMT 2.5% on the dried basis

### SPECIFIC TESTS

- **NITROGEN DETERMINATION, Method I (461):** NLT 2.0% on the dried basis
- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed 10<sup>4</sup> cfu/g; the total combined molds and yeasts count does not exceed 150 cfu/g.
- **LOSS ON DRYING (731)**  
**Analysis:** Dry a sample at 120° for 4 h.  
**Acceptance criteria:** NMT 10%
- **PARTICLE SIZE DISTRIBUTION ESTIMATION BY ANALYTICAL SIEVING (786):** NMT 3% of the total particles exceed 150 µm in size and NMT 20% of the total particles exceed 75 µm in size.
- **FAT CONTENT**  
**Sample:** 4 g from material previously dried under vacuum at 100° for about 5 h  
**Analysis:** Extract the fat from the *Sample* with anhydrous ethyl ether, using a continuous extraction apparatus, the extraction period being 4 h at a condensation rate of 5–6 drops/s. Evaporate the ether from the extract, transfer it to a tared beaker, and dry at 100° to constant weight. Perform a blank determination. Calculate the percentage of fat found, corrected for the blank.  
**Acceptance criteria:** NLT 0.2% on the previously dried basis
- **VISCOSITY—ROTATIONAL METHODS (912)**  
**Sample solution:** Transfer 25 g of Colloidal Oatmeal in small portions, with stirring at 1000 rpm over a 1-min period, to 500 mL of water contained in a beaker, maintained at 45° and equipped with a variable speed mixer. Stir for 5 min after the addition of the last portion of oatmeal. Allow the suspension to stand for 90 min, and equilibrate to ambient temperature. Stir the suspension at 800 rpm for 1 min.  
**Apparatus:** Equip a suitable rotational viscometer with a spindle having a cylinder 1.88 cm in diameter and 6.25 cm high attached to a shaft 0.32 cm in diameter,



the distance from the top of the cylinder to the lower tip of the shaft being 0.75 cm, and the immersion depth being 8.15 cm (No. 1 spindle).

**Analysis:** Determine and record the viscosity of the suspension, with the spindle rotating at 60 rpm. Convert to centipoise by multiplying the reading by the constant for the viscometer spindle and speed employed.

**Acceptance criteria:** The average of three viscosities obtained is greater than 1 and less than 100 centipoises.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.

## Construct Human Fibroblasts in Bilayer Synthetic Scaffold

### DEFINITION

Construct Human Fibroblasts in Bilayer Synthetic Scaffold is a nonliving monolayer skin substitute derived from neonatal foreskins. It is composed of fibroblasts, an extracellular matrix, and a nylon mesh bonded to a transparent, semi-permeable silicone membrane. Human fibroblasts are seeded onto the nylon mesh. The fibroblasts proliferate within the nylon mesh and secrete human matrix proteins. Following freezing, no cellular metabolic activity remains.

The fibroblast-cell banks, from which Construct Human Fibroblasts in Bilayer Synthetic Scaffold is derived, test negative for human and animal viruses and retroviruses, and are also tested for normal cell morphology, human karyology, and isoenzymes. Maternal blood sera are tested for evidence of infection with human immunodeficiency virus types 1 and 2, hepatitis B and C viruses, syphilis, and human T-lymphotropic virus type 1, and are found negative for the purpose of donor selection.

Construct Human Fibroblasts in Bilayer Synthetic Scaffold is manufactured with sterile components under aseptic conditions within the final package. All materials derived from bovine sources originate from countries free of bovine spongiform encephalopathy. During subsequent screening of the fibroblast cell strain at various stages in the manufacturing process, testing for these same viruses, as well as Epstein-Barr virus and human T-lymphotropic virus type 2, is carried out and found to be negative. The final product is inspected and tested to ensure that the product meets specifications.

### SPECIFIC TESTS

#### • HISTOLOGICAL CHARACTERIZATION

**Buffered formalin:** Prepare a solution containing 10% (w/v) formaldehyde solution and 1.0%–1.5% methanol in a suitable buffer, adjusted to a pH of 6.8–7.2.<sup>1</sup>

**Preparation of tissue for staining:** Cut Construct Human Fibroblasts in Bilayer Synthetic Scaffold into 3-mm × 3-mm sections. Place three sections into suitable histological tissue cassettes,<sup>2</sup> and insert the cassettes into suitable histological cassette basket(s).<sup>3</sup>

**Embedding and sectioning:** At a temperature of 40°, sequentially immerse the histological cassette basket(s) in separate solutions of *Buffered formalin* (2 h), two changes of 80% alcohol (30 min/step), alcohol (30 min), three changes of dehydrated alcohol (30 min/step), suitable histological xylene substitute (30 min),<sup>4</sup>

and two changes of suitable xylene substitute (30 min/step). Immerse the histological cassette basket(s) into molten paraffin<sup>5</sup> that is at a temperature of 60° for 30 min. Remove the cassette basket(s), and immerse in a fresh container of molten paraffin at 60° for 60 min. Remove the histological tissue cassette from the container and basket, and disassemble. Fill preheated embedding molds with molten paraffin heated to 56°–60°, and place on top of a preheated warming platform that is designed for histology work. Transfer Construct Human Fibroblasts in Bilayer Synthetic Scaffold specimens from the cassettes using forceps, and place specimens into individual molds. Orient the specimens in molds so as to cut cross-sections. Cool the paraffin by sliding the mold down the platform to its cool side until the paraffin has solidified. Maintain specimen orientation with forceps during cooling, removing the forceps when the paraffin becomes translucent. Slide the paraffin block onto a histological cold plate to rapidly cool the block. Trim the paraffin block with a new single-edged razor blade to form a rectangle or slight trapezoid to within 5 mm of the tissue mass, if necessary. Cool the block at 4° for 15–30 min. Clamp the tissue block into the block holder of the microtome. Fill a histological tissue flotation water bath with fresh water, add an appropriate amount of a suitable histological adhesive,<sup>6</sup> and heat to 5° less than the melting point of the paraffin. Properly mount and adjust the tissue and paraffin block into a microtome. Set the microtome to make cuts 5 µm thick with a blade angle of 5 ± 2°. Insert a sharp stainless steel microtome knife that has been properly honed, or a new disposable microtome knife, into the knife holder. Cut a ribbon that contains 6–10 sections of Construct Human Fibroblasts in Bilayer Synthetic Scaffold. Pick up the ribbon with forceps, and stretch it across the tissue flotation water bath. Separate 2–3 adjacent sections from the ribbon on the water bath. The selected sections should not be compressed, wrinkled, or scratched. Pick up the selected sections by dipping a microscope slide into the water bath under the floating sections, and gently lift the slide out of the water. For each staining procedure, prepare three slides from each of the three starting Construct Human Fibroblasts in Bilayer Synthetic Scaffold 3-mm × 3-mm sections. Allow the mounted sections to air-dry completely, or dry the slide in a 60° oven for 1 h.

#### Hematoxylin–eosin staining

**Hematoxylin–alcohol solution:** Dissolve 2.5 g of hematoxylin in 25.0 mL of dehydrated alcohol with heating.

**Potassium alum solution:** Dissolve 50.0 g of potassium alum in 500 mL of water with heating.

**Hematoxylin staining solution:** Mix *Hematoxylin–alcohol solution* and *Potassium alum solution*. Bring to a boil as rapidly as possible with constant stirring. Do not heat for more than 1 min. Slowly add 0.185 g of sodium iodate. Reheat to a simmer until the solution becomes a deep purple. Remove from heat, and quickly cool. Filter daily before use.

**10% acid alcohol:** Add 5.0 mL of hydrochloric acid to 495 mL of 70% alcohol.

**Eosin solution:** Dissolve 1 g of eosin Y in 100 mL of alcohol. Filter daily before use.

**Analysis:** Sequentially immerse the microscope slide with affixed tissue, as prepared in *Preparation of tissue for staining*, in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), running tap water rinse (1 min), *Hematoxylin staining solution* (4–5 min), running tap water rinse (1 min),

<sup>1</sup> A suitable *Buffered formalin* can be obtained from VWR International, 1310 Goshen Pkwy., West Chester, PA 19380.

<sup>2</sup> A suitable histological tissue cassette can be obtained from Sakura Finetek U.S.A., Inc., 1750 West 214th St., Torrance, CA 90501.

<sup>3</sup> A suitable histological tissue cassette basket can be obtained from Sakura Finetek U.S.A., Inc., 1750 West 214th St., Torrance, CA 90501.

<sup>4</sup> A suitable histological xylene substitute is Citrosolve® Clearing Agent, available from Fisher Scientific, 200 Park Ln., Pittsburgh, PA 15275.

<sup>5</sup> A suitable paraffin for use is Tissue Prep® 2 Embedding Media, available from Fisher Scientific, 200 Park Ln., Pittsburgh, PA 15275.

<sup>6</sup> A suitable histological adhesive for use is HistoSlide® Adhesive, which can be obtained from Poly Scientific Research Corp., 70 Cleveland Ave., Bay Shore, NY 11706-1282.



10% acid alcohol (15 s), running tap water rinse (1 min), a suitable bluing reagent<sup>7</sup> (20–30 s), running tap water rinse (1 min), alcohol (20 s), *Eosin solution* (10–20 s, until a reddish-brown color is obtained in the tissue), three changes of dehydrated alcohol (10 s/step), and three changes of a suitable histological xylene substitute (10 s/step). Adjust the above immersion times as needed to suitably stain the tissue. Remove the slide from the last histological xylene substitute wash, and blot dry the back of the slide. Do not allow the tissue to dry. Affix a coverslip over the tissue using a coverslip mountant.

**Acceptance criteria:** Using USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 1 (hematoxylin–eosin stained)<sup>8</sup> for comparison, the nylon-scaffold mesh, silicone membrane, and secreted collagen-based matrix are present, and the tissue contains normal human fibroblast distributed throughout the secreted matrix, and resembles normal human papillary dermis. The fibroblasts appear elongated and spindle shaped. The tissue contains about 10<sup>6</sup> cells/cm<sup>2</sup> and about 500 cells/mm along the section.

#### Collagen staining

**Bouin's solution:** Mix 75.0 mL of 1.22% picric acid solution, 25.0 L of dimethoxymethane, and 5.0 L of acetic acid.

**Weigert's iron hematoxylin solution A:** Dissolve 1.0 g of hematoxylin in 100 mL of alcohol.

**Weigert's iron hematoxylin solution B:** Mix 4.0 mL of 29% ferric chloride, 95.0 mL of water, and 1.0 mL of hydrochloric acid.

**Weigert's iron hematoxylin working solution:** Combine *Weigert's iron hematoxylin solution A* and *Weigert's iron hematoxylin solution B* (1:1). Pass the solution through a suitable filter of 0.45-μm pore size. Prepare fresh as needed.

**Gomori's trichrome solution:** Mix 1.0 mL of acetic acid and 100 mL of water. Dissolve 0.6 g of chromotrope 2R, 0.3 g of Fast Green FCF, and 0.6 g of phosphotungstic acid.

**1% acetic acid:** Dilute 1 mL of glacial acetic acid with water to make 100 mL of solution.

**Analysis:** Sequentially immerse the microscope slide with affixed tissue, as prepared in *Preparation of tissue for staining*, in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), and running tap water rinse (1 min). Immerse the slide in *Bouin's solution*, and place in a 42° water bath for 1 h. Rinse the slide in water for 10 s. Sequentially immerse the slide in *Weigert's iron hematoxylin working solution* (10 min) and running tap water rinse (10 min). Rinse the slide in water for 10 s, and immerse in *Gomori's trichrome solution* (3–5 min). Rinse the slide in 1% acetic acid for 20 s. Sequentially immerse the slide in three changes of alcohol (10 s/step) and three changes of a suitable histological, aliphatic xylene substitute (10 s/step). Affix a coverslip over the tissue using a suitable coverslip mountant. Nuclei will stain black; cytoplasm, keratin, and muscle fibers will stain red; and collagen and mucin will stain blue.

**Acceptance criteria:** Using USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 2 for comparison, collagen is found throughout the extracellular matrix. The tissue contains normal human fibroblast distributed throughout the secreted matrix and resembles normal human papillary; dermis, muscle fibers, and keratin are absent.

#### Distribution of fibronectin

**Tris-saline buffer:** Combine 0.1 M tris-(hydroxymethyl)aminomethane hydrochloride and 0.15 M sodium chloride, and adjust to a pH of 7.8.

**3% Hydrogen peroxide:** Dilute 30 mL of hydrogen peroxide with water or methanol.

**Diaminobenzidine solution:** Use a suitable solution.<sup>9</sup>

**Hematoxylin staining solution:** Prepare as directed for *Hematoxylin–eosin staining*.

**Analysis:** The microscope slide with affixed tissue as prepared in *Preparation of tissue for staining* is dried either overnight at 37° or for 1 h at 60°. The microscope slide with affixed tissue as prepared in *Preparation of tissue for staining* is sequentially immersed in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), and running tap water rinse (1 min). Sequentially immerse the slide in *Tris-saline buffer* (10 min), 3% hydrogen peroxide (30 min), three changes of *Tris-saline buffer* (1 min/step), a suitable normal rabbit serum<sup>10</sup> (30 min), water (5 min), and three changes of *Tris-saline buffer* (1 min/step). Incubate the slide with a suitable solution of rabbit anti-human fibronectin antibody,<sup>11</sup> diluted using a suitable antibody diluent<sup>12</sup> to an antibody concentration of 21.0 ± 2.1 mg/L for 1 h. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Place enough drops of a biotinylated goat anti-rabbit antibody solution<sup>13</sup> to cover the tissue section, and incubate for 30 min. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Place enough drops of a streptavidin conjugated horseradish peroxidase solution<sup>14</sup> to cover the tissue section, and incubate for 30 min. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Incubate the slide with *Diaminobenzidine solution* for 1–5 min, until a suitable difference in staining is seen by comparison with a control in which the fibronectin (primary) antibody is omitted. Sequentially immerse the slide in water (1 min), *Hematoxylin staining solution* (4–5 min), and water (1 min). Do not allow the tissue to dry. Affix a coverslip over the tissue using a low-viscosity, aqueous, synthetic-resin coverslip mountant.

**Acceptance criteria:** Using USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 3 (diaminobenzidine–hematoxylin stained) for comparison, fibronectin binds to collagen and is found throughout the extracellular matrix. Fibronectin is found colocalizing with the collagen fibers. The intensity of staining may vary from region to region of the slide.

#### • METABOLIC ACTIVITY ASSESSMENT

**DPBS solution A:** Dissolve 1.32 g of calcium chloride and 1.21 g of magnesium sulfate heptahydrate in 2 L of water.

**DPBS solution B:** Dissolve 80.0 g of sodium chloride, 2.0 g of potassium chloride, 11.5 g of dibasic sodium phosphate, 2.0 g of monobasic potassium phosphate, 10.0 g of glucose, 0.36 g of sodium phosphate, 0.5 g of streptomycin sulfate, and 1,000,000 USP Units of penicillin G sodium in 8 L of water.

<sup>9</sup> A suitable Diaminobenzidine solution can be obtained from Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178; catalog number D-6815.

<sup>10</sup> A suitable normal rabbit serum can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

<sup>11</sup> Suitable rabbit anti-human fibronectin antibodies can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

<sup>12</sup> Suitable antibody diluent can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

<sup>13</sup> Suitable biotinylated goat anti-rabbit antibody solution can be obtained from BioGenex, 4600 Norris Canyon Rd., San Ramon, CA 94583.

<sup>14</sup> A suitable streptavidin conjugated horseradish peroxidase solution can be obtained from BioGenex, 4600 Norris Canyon Rd., San Ramon, CA 94583.

<sup>7</sup> A suitable bluing reagent can be obtained from Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178.

<sup>8</sup> These photomicrographs are available as a CD from the USP Reference Standards collection, available to the user through USP Customer Services. To order these and other Reference Standards, call 1-800-227-8772 (U.S. and Canada), +1-301-881-0666 or 00-800-4875-5555 (select Europe); or go online to [www.usp.org](http://www.usp.org). Order item number 1535857.



**DPBS working solution:** Mix *DPBS solution B* and *DPBS solution A* (8:2). Pass the solution through a filter of 0.22- $\mu$ m pore size.

**Dulbecco's modified Eagle's tissue culture medium:** Prepare a solution that contains the components listed in *Table 1*.

Table 1

Component	mg/L
Calcium chloride	264.9
Ferric nitrate nonahydrate	0.10
Potassium chloride	400.0
Magnesium sulfate heptahydrate	200.0
Sodium chloride	6,400.0
Sodium bicarbonate	3,700.0
Sodium phosphate, monobasic (monohydrate)	125.0
Dextrose	4,500.0
Phenol red	15.0
Sodium pyruvate	110.0
L-Arginine hydrochloride	84.0
L-Cystine	48.0
Aminoacetic acid	30.0
L-Histidine hydrochloride monohydrate	42.0
L-Isoleucine	104.8
L-Leucine	104.8
L-Lysine hydrochloride	146.2
L-Methionine	30.0
L-Phenylalanine	66.0
L-Serine	42.0
L-Threonine	95.2
L-Tryptophan	16.0
L-Tyrosine	72.0
L-Valine	93.6
D-Calcium pantothenate	4.0
Choline chloride	4.0
Folic acid	4.0
Inositol	7.0
Nicotinamide	4.0
Pyridoxine hydrochloride	4.0
Riboflavin	0.40
Thiamine hydrochloride	4.0

**L-Glutamine solution:** Prepare 100 mL of a solution containing 29.2 g of L-glutamine.

**Sodium pyruvate solution:** Prepare 100 mL of a solution containing 1.10 g of sodium pyruvate.

**Antibiotic-antimycotic solution:** Prepare 100 mL of a solution containing 0.85 g of sodium chloride, 10,000 USP Units of penicillin G sodium, 10,000  $\mu$ g of streptomycin (base), and 25  $\mu$ g of amphotericin B in water.

**Assay stock medium:** Mix 1000 mL of *Dulbecco's modified Eagle's tissue culture medium*, 10 mL of *L-Glutamine solution*, 10 mL of *Sodium pyruvate solution*, 10 mL of *Antibiotic-antimycotic solution*, and 20 mL of fetal bovine serum.<sup>15</sup>

**MTT-assay solution:** Dissolve 0.50 g of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide in 1 L of *Assay stock medium*, using constant stirring. Sterilize the solution by passing it through a filter of 0.2- $\mu$ m pore size.

**MTT formazan stock solution:** 100  $\mu$ g/mL of 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan in isopropyl alcohol

**MTT formazan calibration solutions:** 15, 30, 45, 60, and 75  $\mu$ g/mL of MTT formazan, using *MTT formazan stock solution* and diluting with isopropyl alcohol

**Analysis:** Thaw Construct Human Fibroblasts in Bilayer Synthetic Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Bilayer Synthetic Scaffold unit. Cut three 11-mm  $\times$  11-mm sections of Construct Human Fibroblasts in Bilayer Synthetic Scaffold, and immerse the sections into separate, 3.0-mL portions of *MTT-assay solution*. Incubate for 2 h at 37  $\pm$  2° in a 3%–7% CO<sub>2</sub>-air environment with shaking on an orbital shaker at 150–200 rpm. After incubation, remove from the 37°, 3%–7% CO<sub>2</sub>-air environment. Remove the *MTT-assay solution*, and rinse twice with *DPBS working solution*. Immerse the Construct Human Fibroblasts in Bilayer Synthetic Scaffold in 2 mL of isopropyl alcohol, and incubate at ambient temperature for 1 h with shaking on an orbital shaker at approximately 125 rpm. Transfer 200- $\mu$ L aliquots of the five *MTT formazan calibration solutions*, in triplicate, and 200- $\mu$ L aliquots of the three isopropyl alcohol extracts of Construct Human Fibroblasts in Bilayer Synthetic Scaffold to a suitable 96-well, flat-bottom plate. Read the absorbance of each aliquot at 540 nm, using 200  $\mu$ L of isopropyl alcohol as the blank.

Plot the responses of the *MTT formazan calibration solutions* versus concentration, in  $\mu$ g of MTT formazan/mL, and calculate the regression line using the least-squares method.

**System suitability requirements:** The test is considered valid if the regression line has a square of the correlation coefficient NLT 0.95.

**Acceptance criteria:** The absorbance value of individual Construct Human Fibroblasts in Bilayer Synthetic Scaffold sections at 540 nm is less than 0.1.

#### • DNA CONTENT

**Cell culture water:** Sterile water containing NMT 0.005 USP Endotoxin Unit/mL

**DNA extraction buffer:** Transfer 850 mL of *Cell culture water* to a sterile, 1-L graduated container. Dissolve 12.110 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 3.802 g of edetate disodium, 23.380 g of sodium chloride, and 0.080 g of sodium dodecyl sulfate, with stirring. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 7.0. Dilute with *Cell culture water* to 1 L.

**Proteinase K solution:** Prepare a solution of Tritirachium album proteinase K in 10 mM of 2-amino-2-hydroxymethyl-1,3-propanediol, adjusted to a pH of 7.5, having an activity of 600 units/mL.<sup>16</sup>

**Working DNA extraction buffer:** Add 1.22 mL of *Proteinase K solution* to 38.78 mL of *DNA extraction buffer*, and mix.

**Dilution buffer:** Transfer 850 mL of *Cell culture water* to a sterile, 1-L graduated container. Add 1.211 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 3.802 g of edetate disodium, and 5.844 g of sodium chloride, with stirring. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 7.0. Dilute with *Cell culture water* to 1 L.

**DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution:** Prepare a solution containing 8.00 g/L of sodium chloride, 1.15 g/L of dibasic sodium phosphate (anhydrous), 0.20 g/L of potassium chloride, and 0.20 g/L of monobasic potassium phosphate in water.

**Calf thymus DNA solution:** Prepare a solution containing 1 mg/L of calf thymus DNA in *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*, mixing thoroughly for 12–24 h at ambient temperature. Dilute the resulting solution with *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* to prepare a solution containing 50  $\mu$ g/mL of calf thymus DNA, mixing thoroughly on a vortex mixer for 10 min.

<sup>15</sup> A suitable fetal bovine serum can be obtained from HyClone, 925 West 1800 South, Logan, UT 84321; catalog number SH30070.03.

<sup>16</sup> A suitable Proteinase K solution can be obtained from Roche Diagnostics Corp., Roche Applied Sciences, P.O. Box 50414, 9115 Hague Rd., Indianapolis, IN 46250-0414.



**Calf thymus DNA calibration solutions:** Prepare four calibration solutions containing 5, 10, 15, and 20 µg/mL of calf thymus DNA, using *Calf thymus DNA solution*, and diluting with *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*.

**DNA staining solution:** Prepare a solution containing 0.5 µg of 2'-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi(1H-benzimidazole) trihydrochloride pentahydrate/mL of *Dilution buffer*. Store in low-actinic glassware.

**Analysis:** Thaw Construct Human Fibroblasts in Bilayer Synthetic Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Bilayer Synthetic Scaffold unit. Cut three 11-mm × 11-mm sections of Construct Human Fibroblasts in Bilayer Synthetic Scaffold. To each of three microcentrifuge tubes add 1 mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*. Immerse a single Construct Human Fibroblasts in Bilayer Synthetic Scaffold 11-mm × 11-mm section into each microcentrifuge tube to remove the cryopreservative. Aspirate the *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* from each tube, and replace with 1 mL of *Working DNA extraction buffer*, making sure that each Construct Human Fibroblasts in Bilayer Synthetic Scaffold is completely submerged in the extraction buffer. Incubate the samples in a 56°–60° water bath for 4–18 h. Sonicate for 10–15 s using an ultrasonic cell disrupter to achieve complete cellular disruption of the tissue and to mix the contents of the tube. Centrifuge the microcentrifuge tubes at 12,000–15,000 × g to pellet non-DNA material. Transfer three 50-µL aliquots of each sample supernatant to individual wells of a 96-well black plate suitable for performing fluorescent analysis. Transfer triplicate 50-µL aliquots of each of the *Calf thymus DNA calibration solutions* to the 96-well plate, as well as a 50-µL aliquot of *DPBS working solution* for the blank. Add 150 µL of *DNA staining solution* to all wells containing the tissue samples, *Calf thymus DNA calibration solutions*, and the blank. Cover with aluminum foil, and place in a dark cabinet for 30–45 min at 15°–30°. Read the fluorescence of each well, using an excitation wavelength of 355 nm and an emission wavelength of 460 nm, blanking against the *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* well. Plot the responses of the *Calf thymus DNA calibration solutions* versus concentration, in µg/mL of calf thymus DNA, and calculate the regression line using the least-squares method. The test is considered valid if the %CV of the replicate values is less than 15%, the slope is 4.48–6.27, the y-intercept is between –2.04 and 3.65, and the square of the correlation coefficient is NLT 0.990. From the regression line so obtained, determine the amount of DNA, in µg/11-mm × 11-mm sample.

**Acceptance criteria:** The amount of DNA of an individual Construct Human Fibroblasts in Bilayer Synthetic Scaffold 11-mm × 11-mm section is between 6 and 14 µg.

#### • TOTAL COLLAGEN CONTENT

**DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution:** Proceed as directed for *DNA Content*.

**DPBS with Ca<sup>++</sup>, Mg<sup>++</sup> solution:** 8.00 mg/mL of sodium chloride, 1.15 mg/mL of dibasic sodium phosphate (anhydrous), 0.20 mg/mL of potassium chloride, 0.20 mg/mL of monobasic potassium phosphate, 0.10 mg/mL of magnesium chloride hexahydrate, and 0.10 mg/mL of calcium chloride (anhydrous) in water

**Collagenase extraction solution:** At least 250 Units/mL of *Clostridium histolyticum* collagenase, type 2, in *DPBS with Ca<sup>++</sup>, Mg<sup>++</sup> solution*

**2% Acetic acid solution:** Mix 10 mL of acetic acid with 490 mL of water.

**Collagen standard stock solution:** 2 mg/mL of collagen, type I, in 2% *Acetic acid solution*

**Collagen calibration standards:** Cut polyglactin mesh<sup>17</sup> into seventeen 11-mm × 11-mm squares, and place one square into 17 individual wells of a 24-well plate. Each well of the 24-well plate has a surface area of 220 mm<sup>2</sup> and a volume of 3.5 mL. In quadruplicate, prepare wells containing 0.050, 0.100, 0.200, and 0.400 mg of collagen by adding 25, 50, 100, and 200 µL, respectively, of the *Collagen standard stock solution*. The remaining well to which no *Collagen standard stock solution* has been added is used as the blank. Allow the wells to air dry.

**Sirius red solution:** 1 mg/mL of Direct Red 80 in saturated picric acid

**1% (p-tert-Octylphenoxy) polyethoxyethanol solution:** Mix 10 mL of (p-tert-Octylphenoxy) polyethoxyethanol in 990 mL of *DPBS with Ca<sup>++</sup>, Mg<sup>++</sup> solution*.

**Analysis:** Thaw Construct Human Fibroblasts in Bilayer Synthetic Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Bilayer Synthetic Scaffold unit. Cut three 11-mm × 11-mm sections of Construct Human Fibroblasts in Bilayer Synthetic Scaffold. Place each test section into separate wells of a 24-well plate. Add 200 µL of 1% (p-tert-Octylphenoxy) polyethoxyethanol solution to each sample. Shake on a rotating platform shaker at 100–150 rpm for 60–70 min at room temperature. Aspirate off the 1% (p-tert-Octylphenoxy) polyethoxyethanol solution, and rinse three times with 2 mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*. Transfer each of the collagen standards to individual wells of the 24-well plate. Add 0.5 mL of *Sirius red solution* to each test sample and collagen standards. Shake on a rotating platform shaker at 100–150 rpm for 60 min at room temperature. Aspirate off the *Sirius red solution* from each well. Rinse each well twice with 2 mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*. Add an additional 2 mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* to each well, and allow to stand for 2 min. Aspirate off the *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*, and rinse twice more with 2 mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*. Aspirate off all traces of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*. Add 0.5 mL of *Collagenase extraction solution* to each well containing the *Collagen calibration standards*. Add 2.0 mL of *Collagenase extraction solution* to each well containing test samples. Rotate the plate on an orbital rotator at 150 rpm for 90 min at 37°. Transfer 200 µL from each well, to a suitable 96-well, flat-bottom plate. Read the absorbance of each aliquot at 540 nm. Dilute the Construct Human Fibroblasts in Bilayer Synthetic Scaffold sample preparation further with *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* if the absorbance is greater than the absorbance of the highest of the *Collagen calibration standards*.

Plot the responses of the *Collagen calibration standards* versus the amount, in mg of collagen, and calculate the regression line using the least-squares method.

**System suitability requirements:** The test is considered valid if the slope is 3.00–5.00 and the square of the correlation coefficient is ≥ 0.950.

Determine the collagen content, in mg, of an 11- × 11-mm section of Construct Human Fibroblasts in Bilayer Synthetic Scaffold from the regression line, and by using the following equation:

$$\text{Result} = D \times A \times SC_{SR}$$

- $D$  = dilution factor (normally 4, unless the sample had to be further diluted)  
 $A$  = absorbance at 540 nm  
 $SC_{SR}$  = slope of the regression line of the standards calculated above

<sup>17</sup> A suitable polyglactin mesh can be obtained from Ethicon Co., Johnson & Johnson Corp., 425 Hoes Ln., P.O. Box 6800, Piscataway, NJ 08855.



**Acceptance criteria:** The amount of collagen in individual Construct Human Fibroblasts in Bilayer Synthetic Scaffold 11- × 11-mm samples is 0.50–4.0 mg.

• **BACTERIAL ENDOTOXINS TEST (85)**

**Sample solution:** Thaw Construct Human Fibroblasts in Bilayer Synthetic Scaffold by placing the tissue, still in its polycarbonate cassette contained in a plastic covering bag, in a water bath heated to a maximum of 37° for 15–20 min until no visible ice remains in the cassette. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Bilayer Synthetic Scaffold unit.

**Analysis:** Remove the unit from the polycarbonate cassette, and immerse in 25 mL of LAL Reagent Water. Extract for 60 min at 37° with shaking on an orbital shaker set at 125 revolutions/min. Remove a 4-mL aliquot of the extract for testing.

**Acceptance criteria:** NMT 0.5 USP Endotoxin Unit/mL

• **STERILITY TESTS (71)**

**Sample solution:** Thaw Construct Human Fibroblasts in Bilayer Synthetic Scaffold by placing the tissue, still in its polycarbonate cassette contained in a plastic covering bag, in a water bath heated to a maximum of 37° for 15–20 min until no visible ice remains in the cassette. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Bilayer Synthetic Scaffold unit.

**Analysis:** Perform the test on 20 mL of the cryopreservative.

**Acceptance criteria:** Meets the requirements

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Construct Human Fibroblasts in Bilayer Synthetic Scaffold is aseptically packaged and supplied frozen in a clear plastic cassette containing two, approximately 12.5- × 19-cm units. The solution within the cassette is a phosphate-buffered cryoprotectant solution used to facilitate long-term storage. A clear plastic bag surrounds the cassette for its protection. Construct Human Fibroblasts in Bilayer Synthetic Scaffold should be stored at a temperature of –70° to –20° for no longer than 18 months.

• **LABELING:** The label indicates the dimensions of the Construct Human Fibroblasts in Bilayer Synthetic Scaffold material enclosed. It contains the expiry date, required storage conditions, and the lot number. The label cautions that Construct Human Fibroblasts in Bilayer Synthetic Scaffold is not to be used if the package shows signs of damage. Additional labeling requirements include instructions on the proper thawing and handling of Construct Human Fibroblasts in Bilayer Synthetic Scaffold and the time frame for use after package opening.

• **USP REFERENCE STANDARDS, Authentic Visual References (11):** USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrographs. These three photomicrographs represent examples of passing units, prepared as directed in *Hematoxylin–eosin staining*, *Collagen staining*, and *Distribution of fibronectin*. They are specified to assist in ascertaining histological quality. The fibroblasts are embedded in an extracellular matrix that they have secreted (USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 1). The collagen (USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 2) and fibronectin (USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 3) are to be found throughout the extracellular matrix. The nylon fibers (yellow in USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 1) and the silicone backing (gray in USP Construct Human Fibroblasts in Bilayer Synthetic Scaffold Reference Photomicrograph 1) are frequently visible, although easily lost during processing.

• **USP REFERENCE STANDARDS (11)**  
USP Endotoxin RS

## Construct Human Fibroblasts in Polyglactin Scaffold

### DEFINITION

Construct Human Fibroblasts in Polyglactin Scaffold is a living monolayer skin substitute derived from neonatal foreskins. It is composed of fibroblasts, an extracellular matrix, and a bioabsorbable scaffold. Human fibroblasts are seeded onto a bioabsorbable, nonantigenic and nonpyrogenic mesh scaffold composed of polyglactin, a copolymer of glycolide and lactide. The fibroblast-cell banks, from which Construct Human Fibroblasts in Polyglactin Scaffold are derived have passed applicable donor eligibility requirements for relevant communicable diseases. Maternal blood sera are tested for evidence of infection with human immunodeficiency virus types 1 and 2, hepatitis B and C viruses, syphilis, and human T-lymphotropic virus type 1 and are found negative for the purpose of donor selection. Construct Human Fibroblasts in Polyglactin Scaffold is manufactured with sterile components under aseptic conditions within the final package. Reagents used in the manufacture of Construct Human Fibroblasts in Polyglactin Scaffold are tested and found free of viruses, retroviruses, endotoxins, and mycoplasma before use. All materials derived from bovine sources originate from countries free of bovine spongiform encephalopathy. During subsequent screening of the fibroblast cell strain at various stages in the manufacturing process, testing for these same viruses, as well as Epstein-Barr virus and human T-lymphotropic virus type 2, is carried out and found to be negative. The final product is inspected and tested to ensure that the product meets specifications.

### SPECIFIC TESTS

• **HISTOLOGICAL EVALUATION**

**Buffered formalin:** Prepare a solution containing 10% (w/v) formaldehyde solution and 1.0%–1.5% methanol in a suitable buffer, adjusted to a pH of 6.8–7.2.<sup>1</sup>

**Preparation of tissue for staining:** Cut Construct Human Fibroblasts in Polyglactin Scaffold into 3-mm × 3-mm sections. Place three sections into suitable histological tissue cassettes,<sup>2</sup> and insert the cassettes into suitable histological cassette basket(s).<sup>3</sup> At a temperature of 40°, sequentially immerse the histological cassette basket(s) in separate solutions of *Buffered formalin* (2 h), two changes of 80% alcohol (30 min/step), alcohol (30 min), three changes of dehydrated alcohol (30 min/step), suitable histological xylene substitute (30 min),<sup>4</sup> and two changes of suitable xylene substitute (30 min/step). Immerse the histological cassette basket(s) into molten paraffin<sup>5</sup> that is at a temperature of 60° for 30 min. Remove the cassette basket(s), and immerse in a fresh container of molten paraffin at 60° for 60 min. Remove the histological tissue cassette from the container and basket, and disassemble. Fill preheated embedding molds with molten paraffin heated to 56°–60°, and place on top of a preheated warming platform that is designed for histology work. Transfer Construct Human Fibroblasts in Polyglactin

<sup>1</sup> A suitable *Buffered formalin* can be obtained from VWR International, 1310 Goshen Pkwy., West Chester, PA 19380.

<sup>2</sup> A suitable histological tissue cassette can be obtained from Sakura Finetek U.S.A., Inc., 1750 West 214th St., Torrance, CA 90501.

<sup>3</sup> A suitable histological tissue cassette basket can be obtained from Sakura Finetek U.S.A., Inc., 1750 West 214th St., Torrance, CA 90501.

<sup>4</sup> A suitable histological xylene substitute is Citrosolve® Clearing Agent, available from Fisher Scientific, 200 Park Ln., Pittsburgh, PA 15275.

<sup>5</sup> A suitable paraffin for use is Tissue Prep® 2 Embedding Media, available from Fisher Scientific, 200 Park Ln., Pittsburgh, PA 15275.



Scaffold specimens from the cassettes using forceps, and place specimens into individual molds. Orient the specimens in molds so as to cut cross-sections. Cool the paraffin by sliding the mold down the platform to its cool side until the paraffin has solidified. Maintain specimen orientation with forceps during cooling, removing the forceps when the paraffin becomes translucent. Slide the paraffin block onto a histological cold plate to rapidly cool the block. Trim the paraffin block with a new single-edged razor blade to form a rectangle or slight trapezoid to within 5 mm of the tissue mass, if necessary. Cool the block at 4° for 15–30 min. Clamp the tissue block into the block holder of the microtome. Fill a histological tissue flotation water bath with fresh water, add an appropriate amount of a suitable histological adhesive,<sup>6</sup> and heat to 5° less than the melting point of the paraffin. Properly mount and adjust the tissue and paraffin block into a microtome. Set the microtome to make cuts 5-μm thick with a blade angle of 5 ± 2°. Insert a sharp stainless steel microtome knife that has been properly honed or a new disposable microtome knife into the knife holder. Cut a ribbon that contains 6–10 sections of Construct Human Fibroblasts in Polyglactin Scaffold. Pick up the ribbon with forceps, and stretch it across the tissue flotation water bath. Separate 2–3 adjacent sections from the ribbon on the water bath. The selected sections should not be compressed, wrinkled, or scratched. Pick up the selected sections by dipping a microscope slide into the water bath under the floating sections, and gently lift the slide out of the water. For each staining procedure, prepare three slides from each of the three starting Construct Human Fibroblasts in Polyglactin Scaffold 3-mm × 3-mm sections. Allow the mounted sections to air-dry completely, or dry the slide in a 60° oven for 1 h.

#### Hematoxylin–eosin staining

**Hematoxylin–alcohol solution:** Dissolve 2.5 g of hematoxylin in 25.0 mL of dehydrated alcohol with heating.

**Potassium alum solution:** Dissolve 50.0 g of potassium alum in 500 mL of water with heating.

**Hematoxylin staining solution:** Mix *Hematoxylin–alcohol solution* and *Potassium alum solution*. Bring to a boil as rapidly as possible with constant stirring. Do not heat for more than 1 min. Slowly add 0.185 g of sodium iodate. Reheat to a simmer until the solution becomes a deep purple. Remove from heat, and quickly cool. Filter daily before use.

**10% Acid alcohol:** Add 5.0 mL of hydrochloric acid to 495 mL of 70% alcohol.

**Eosin solution:** Dissolve 1 g of eosin Y in 100 mL of alcohol. Filter daily before use.

**Analysis:** Sequentially immerse the microscope slide with affixed tissue, as prepared in *Preparation of tissue for staining*, in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), running tap water rinse (1 min), *Hematoxylin staining solution* (4–5 min), running tap water rinse (1 min), *10% Acid alcohol* (15 s), running tap water rinse (1 min), a suitable bluing reagent<sup>7</sup> (20–30 s), running tap water rinse (1 min), alcohol (20 s), *Eosin solution* (10–20 s, until a reddish-brown color is obtained in the tissue), three changes of dehydrated alcohol (10 s/step), and three changes of a suitable histological xylene substitute (10 s/step). Adjust the above immersion times as needed to suitably stain the tissue. Remove the slide from the last histological xylene substitute wash, and blot dry the back of the slide. Do

not allow the tissue to dry. Affix a coverslip over the tissue using a coverslip mountant.

**Acceptance criteria:** Using USP Construct Human Fibroblasts in Polyglactin Scaffold Reference Photomicrograph 1<sup>8</sup> for comparison, the test article shows a polyglactin scaffold mesh and a secreted collagen-based matrix; the tissue contains normal human fibroblast distributed throughout the secreted matrix and resembles normal human papillary dermis. Fibroblasts appear elongated and spindle-shaped. The tissue, which is 100–300 μm thick, contains 10<sup>6</sup> cells/cm<sup>2</sup>.

#### Collagen staining

**Bouin's solution:** Mix 75 mL of 1.22% picric acid solution, 25 L of dimethoxymethane, and 5.0 L of acetic acid.

**Weigert's iron hematoxylin solution A:** Dissolve 1 g of hematoxylin in 100 mL of alcohol.

**Weigert's iron hematoxylin solution B:** Mix 4.0 mL of 29% ferric chloride, 95.0 mL of water, and 1.0 mL of hydrochloric acid.

**Weigert's iron hematoxylin working solution:** Mix equal volumes of *Weigert's iron hematoxylin solution A* and *Weigert's iron hematoxylin solution B*. Pass the solution through a filter of 0.45-μm pore size. Prepare fresh as needed.

**Gomori's trichrome solution:** Mix 1.0 mL of acetic acid and 100 mL of water. Dissolve 0.6 g of chromotrope 2R, 0.3 g of Fast Green FCF, and 0.6 g of phosphotungstic acid.

**1% Acetic acid:** Dilute 1 mL of glacial acetic acid with water to make 100 mL of solution.

**Analysis:** The microscope slide with affixed tissue, as prepared in *Preparation of tissue for staining*, is sequentially immersed in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), and running tap water rinse (1 min). Immerse the slide in *Bouin's solution*, and place in a 42° water bath for 1 h. Rinse the slide in water for 10 s. Sequentially immerse the slide in *Weigert's iron hematoxylin working solution* (10 min) and running tap water rinse (10 min). Rinse the slide in water for 10 s, and immerse in *Gomori's trichrome solution* (3–5 min). Rinse the slide in *1% acetic acid* for 20 s. Sequentially immerse the slide in three changes of alcohol (10 s/step) and three changes of a suitable histological, aliphatic xylene substitute (10 s/step). Affix a coverslip over the tissue using a suitable coverslip mountant. Nuclei will stain black; cytoplasm, keratin, and muscle fibers will stain red; and collagen and mucin will stain blue.

**Acceptance criteria:** Using USP Construct Human Fibroblasts in Polyglactin Scaffold Reference Photomicrograph 2 for comparison, the tissue contains normal human fibroblast distributed throughout the secreted matrix and resembles normal human papillary dermis; muscle fibers, and keratin are absent.

#### Distribution of fibronectin

**Tris-saline buffer:** Prepare a solution containing 0.1 M tris-(hydroxymethyl)aminomethane hydrochloride and 0.15 M sodium chloride, adjusted to a pH of 7.8.

**3% Hydrogen peroxide:** Dilute 30 mL of hydrogen peroxide with water or methanol.

**Diaminobenzidine solution:** Use a suitable solution.<sup>9</sup>

**Hematoxylin staining solution:** Prepare as directed for *Hematoxylin–eosin staining*.

**Analysis:** The microscope slide with affixed tissue as prepared in *Preparation of tissue for staining* is dried either overnight at 37° or for 1 h at 60°. The micro-

<sup>6</sup> A suitable histological adhesive for use is Histoslide® Adhesive, which can be obtained from Poly Scientific Research Corp., 70 Cleveland Ave., Bay Shore, NY 11706-1282.

<sup>7</sup> A suitable bluing reagent can be obtained from Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178.

<sup>8</sup> These photomicrographs are available as a CD from the USP Reference Standards collection, available to the user through USP Customer Services. To order these and other Reference Standards, call 1-800-227-8772 (U.S. and Canada), +1-301-881-0666 or 00-800-4875-5555 (select Europe); or go online to [www.usp.org](http://www.usp.org). Order item number 1535868.

<sup>9</sup> A suitable Diaminobenzidine solution can be obtained from Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178; catalog number D-6815.



scope slide with affixed tissue as prepared in *Preparation of tissue for staining* is sequentially immersed in three changes of a suitable histological, aliphatic xylene substitute (2 min/step), three changes of dehydrated alcohol (1 min/step), alcohol (20 s), and running tap water rinse (1 min). Sequentially immerse the slide in *Tris-saline buffer* (10 min), 3% hydrogen peroxide (30 min), three changes of *Tris-saline buffer* (1 min/step), a suitable normal rabbit serum<sup>10</sup> (30 min), water (5 min), and three changes of *Tris-saline buffer* (1 min/step). Incubate the slide with a suitable solution of rabbit anti-human fibronectin antibody,<sup>11</sup> diluted using a suitable antibody diluent<sup>12</sup> to an antibody concentration of  $21.0 \pm 2.1$  mg/L for 1 h. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Place enough drops of a biotinylated goat anti-rabbit antibody solution<sup>13</sup> to cover the tissue section, and incubate for 30 min. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Place enough drops of a streptavidin conjugated horseradish peroxidase solution<sup>14</sup> to cover the tissue section, and incubate for 30 min. Sequentially immerse the slide in water (5 min) and three changes of *Tris-saline buffer* (1 min/step). Incubate the slide with *Diaminobenzidine solution* for 1–5 min, until a suitable difference in staining is seen by comparison with a control in which the fibronectin (primary) antibody is omitted. Sequentially immerse the slide in water (1 min), *Hematoxylin staining solution* (4–5 min), and water (1 min). Do not allow the tissue to dry. Affix a coverslip over the tissue using a low-viscosity, aqueous, synthetic-resin coverslip mountant.

**Acceptance criteria:** Using USP Construct Human Fibroblasts in Polyglactin Scaffold Reference Photomicrograph 3 for comparison, fibronectin is found colocalizing with the collagen fibers. The intensity of staining may vary from region to region of the slide.

#### • METABOLIC ACTIVITY ASSESSMENT

**DPBS solution A:** Dissolve 1.32 g of calcium chloride and 1.21 g of magnesium sulfate heptahydrate in 2 L of water.

**DPBS solution B:** Dissolve 80.0 g of sodium chloride, 2.0 g of potassium chloride, 11.5 g of dibasic sodium phosphate, 2.0 g of monobasic potassium phosphate, 10.0 g of glucose, 0.36 g of sodium phosphate, 0.5 g of streptomycin sulfate, and 1,000,000 USP Units of penicillin G sodium in 8 L water.

**DPBS working solution:** Mix *DPBS solution B* with *DPBS solution A* (8:2). Pass the solution through a filter of 0.22- $\mu$ m pore size.

**Dulbecco's modified Eagle's tissue culture medium:** Prepare a solution that contains the components listed in *Table 1*.

Table 1

Component	mg/L
Calcium chloride	264.9
Ferric nitrate nonahydrate	0.10
Potassium chloride	400.0
Magnesium sulfate heptahydrate	200.0
Sodium chloride	6,400.0
Sodium bicarbonate	3,700.0
Sodium phosphate, monobasic (monohydrate)	125.0

<sup>10</sup> A suitable normal rabbit serum can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

<sup>11</sup> Suitable rabbit anti-human fibronectin antibodies can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

<sup>12</sup> Suitable antibody diluent can be obtained from Dako Corp., 6392 Via Real, Carpinteria, CA 93013.

<sup>13</sup> Suitable biotinylated goat anti-rabbit antibody solution can be obtained from BioGenex, 4600 Norris Canyon Rd., San Ramon, CA 94583.

<sup>14</sup> A suitable streptavidin conjugated horseradish peroxidase solution can be obtained from BioGenex, 4600 Norris Canyon Rd., San Ramon, CA 94583.

Table 1 (Continued)

Component	mg/L
Dextrose	4,500.0
Phenol red	15.0
Sodium pyruvate	110.0
L-Arginine hydrochloride	84.0
L-Cystine	48.0
Aminoacetic acid	30.0
L-Histidine hydrochloride monohydrate	42.0
L-Isoleucine	104.8
L-Leucine	104.8
L-Lysine hydrochloride	146.2
L-Methionine	30.0
L-Phenylalanine	66.0
L-Serine	42.0
L-Threonine	95.2
L-Tryptophan	16.0
L-Tyrosine	72.0
L-Valine	93.6
D-Calcium pantothenate	4.0
Choline chloride	4.0
Folic acid	4.0
Inositol	7.0
Nicotinamide	4.0
Pyridoxine hydrochloride	4.0
Riboflavin	0.40
Thiamine hydrochloride	4.0

**L-Glutamine solution:** Prepare a 100-mL solution containing 2.92 g of L-glutamine.

**Sodium pyruvate solution:** Prepare 100 mL of a solution containing 1.10 g of sodium pyruvate.

**Antibiotic-antimycotic solution:** Prepare 100 mL of a solution containing 0.85 g of sodium chloride, 10,000 USP Units of penicillin G sodium, 10,000  $\mu$ g of streptomycin (base), and 25  $\mu$ g of amphotericin B.

**Assay stock medium:** Mix 1000 mL of *Dulbecco's modified Eagle's tissue culture medium*, 10 mL of *L-Glutamine solution*, 10 mL of *Sodium pyruvate solution*, 10 mL of *Antibiotic-antimycotic solution*, and 20 mL of fetal bovine serum.<sup>15</sup>

**MTT-assay solution:** Dissolve 0.50 g of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide in 1 L of *Assay stock medium*, using constant stirring. Sterilize the solution by passing it through a filter of 0.2- $\mu$ m pore size.

**MTT formazan stock solution:** 100  $\mu$ g/mL of 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan in isopropyl alcohol

**MTT formazan calibration solutions:** Prepare calibration solutions of 15, 30, 45, 60, and 75  $\mu$ g/mL of MTT formazan, using *MTT formazan stock solution* and diluting with isopropyl alcohol.

**Analysis:** Thaw Construct Human Fibroblasts in Polyglactin Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Polyglactin Scaffold unit. Cut three 11-mm  $\times$  11-mm sections of Construct Human Fibroblasts in Polyglactin Scaffold, and immerse the sections into separate, 3.0 mL portions of *MTT-assay solution*. Incubate for 2 h at  $37 \pm 2^\circ$  in a 3%–7% CO<sub>2</sub>-air environment with shaking on an orbital shaker at 150–200 rpm. After incubation remove from the 37°, 3%–7% CO<sub>2</sub>-air environment. Remove the *MTT-assay solution*, and rinse twice with *DPBS working solution*. Immerse the Construct Human Fibroblasts in Polyglactin Scaffold in 2 mL

<sup>15</sup> A suitable fetal bovine serum can be obtained from HyClone, 925 West 1800 South, Logan, UT 84321; catalog number SH30070.03.



of isopropyl alcohol, and incubate at ambient temperature for 1 h with shaking on an orbital shaker at approximately 125 rpm. Transfer 200- $\mu$ L aliquots of the five *MTT formazan calibration solutions*, in triplicate, and 200- $\mu$ L aliquots of the three isopropyl alcohol extracts of Construct Human Fibroblasts in Polyglactin Scaffold to a suitable 96-well flat-bottom plate. Read the absorbance of each aliquot at 540 nm, using 200  $\mu$ L of isopropyl alcohol as the blank. Plot the responses of the *MTT formazan calibration solutions* versus concentration, in  $\mu$ g/mL of MTT formazan, and calculate the regression line using the least-squares method.

**System suitability:** The test is considered valid if the regression line has a square of the correlation coefficient NLT 0.95.

**Acceptance criteria:** The absorbance value of individual, thawed Construct Human Fibroblasts in Polyglactin Scaffold sections at 540 nm is between 0.30 and 0.86.

#### • DNA CONTENT

**Cell culture water:** Sterile water containing NMT 0.005 USP Endotoxin Unit/mL.

**DNA extraction buffer:** Transfer 850 mL of *Cell culture water* to a sterile, 1-L graduated container. Dissolve 12.110 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 3.802 g of edetate disodium, 23.380 g of sodium chloride, and 0.080 g of sodium dodecyl sulfate, with stirring. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 7.0. Dilute with *Cell culture water* to 1 L.

**Proteinase K solution:** Prepare a solution of *Trinitirachium album* proteinase K in 10 mM of 2-amino-2-hydroxymethyl-1,3-propanediol, adjusted to a pH of 7.5, having an activity of 600 units/mL.<sup>16</sup>

**Working DNA extraction buffer:** Add 1.22 mL of *Proteinase K solution* to 38.78 mL of *DNA extraction buffer* and mix.

**Dilution buffer:** Transfer 850 mL of *Cell culture water* to a sterile, 1-L graduated container. Add 1.211 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 3.802 g of edetate disodium, and 5.844 g of sodium chloride, with stirring. Adjust with 1 N hydrochloric acid or 1 N sodium hydroxide to a pH of 7.0. Dilute with *Cell culture water* to 1 L.

**DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution:** Prepare a solution containing 8.00 g/L of sodium chloride, 1.15 g/L of dibasic sodium phosphate (anhydrous), 0.20 g/L of potassium chloride, and 0.20 g/L of monobasic potassium phosphate in water.

**Calf thymus DNA solution:** Prepare a solution containing 1 mg/L of calf thymus DNA in *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*, mixing thoroughly for 12–24 h at ambient temperature. Dilute the resulting solution with *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* to prepare a solution containing 50  $\mu$ g/mL of calf thymus DNA, mixing thoroughly on a vortex mixer for 10 min.

**Calf thymus DNA calibration solutions:** Prepare four calibration solutions containing 5, 10, 15, and 20  $\mu$ g/mL of calf thymus DNA, using *Calf thymus DNA solution* and diluting with *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*.

**DNA staining solution:** Prepare a solution containing 0.5  $\mu$ g of 2'-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi(1H-benzimidazole) trihydrochloride pentahydrate/mL of *Dilution buffer*. Store in low-actinic glassware.

**Analysis:** Thaw Construct Human Fibroblasts in Polyglactin Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Polyglactin Scaffold unit. Cut three 11-mm  $\times$  11-mm sections of Construct Human Fibroblasts in Polyglactin Scaffold. To each of three microcentrifuge

tubes add 1 mL of *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution*. Immerse a single Construct Human Fibroblasts in Polyglactin Scaffold 11-mm  $\times$  11-mm section into each microcentrifuge tube to remove the cryopreservative. Aspirate the *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* from each tube, and replace with 1 mL of *Working DNA extraction buffer*, making sure that each Construct Human Fibroblasts in Polyglactin Scaffold is completely submerged in the extraction buffer. Incubate the samples in a 56°–60° water bath for 4–18 h. Sonicate for 10–15 s using an ultrasonic cell disrupter to achieve complete cellular disruption of the tissue and to mix the contents of the tube. Centrifuge the microcentrifuge tubes at 12,000–15,000  $\times$  g to pellet non-DNA material. Transfer three 50- $\mu$ L aliquots of each sample supernatant to individual wells of a 96-well black plate suitable for performing fluorescent analysis. Transfer triplicate 50- $\mu$ L aliquots of each of the *Calf thymus DNA calibration solutions* to the 96-well plate, as well as a 50- $\mu$ L aliquot of *DPBS working solution* for the blank. Add 150  $\mu$ L of *DNA staining solution* to all wells containing the tissue samples, *Calf thymus DNA calibration solutions*, and the blank. Cover with aluminum foil, and place in a dark cabinet for 30–45 min at 15°–30°. Read the fluorescence of each well, using an excitation wavelength of 355 nm and an emission wavelength of 460 nm, blanking against the *DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution* well. Plot the responses of the *Calf thymus DNA calibration solutions* versus concentration, in  $\mu$ g/mL of calf thymus DNA, and calculate the regression line using the least-squares method. From the regression line so obtained, determine the amount of DNA in  $\mu$ g/11-mm  $\times$  11-mm sample.

**System suitability requirements:** The test is considered valid if the %CV of the replicate values is less than 15%, the slope is between 4.48 and 6.27, the y-intercept is between –2.04 and 3.65, and the square of the correlation coefficient is NLT 0.990.

**Acceptance criteria:** The amount of DNA of an individual Construct Human Fibroblasts in Polyglactin Scaffold 11-mm  $\times$  11-mm section is between 6 and 15  $\mu$ g.

#### • TOTAL COLLAGEN CONTENT

**DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution:** Prepare as directed for *DNA Content*.

**DPBS with Ca<sup>++</sup>, Mg<sup>++</sup> solution:** 8.00 mg/mL of sodium chloride, 1.15 mg/mL of dibasic sodium phosphate (anhydrous), 0.20 mg/mL of potassium chloride, and 0.20 mg/mL of monobasic potassium phosphate, 0.10 mg/mL of magnesium chloride hexahydrate, and 0.10 mg/mL of calcium chloride (anhydrous) in water.

**Collagenase extraction solution:** At least 250 Units/mL of *Clostridium histolyticum* collagenase, type 2, in *DPBS with Ca<sup>++</sup>, Mg<sup>++</sup> solution*.

**2% Acetic acid solution:** Mix 10 mL of acetic acid with 490 mL of water.

**Collagen standard stock solution:** 2 mg/mL of collagen, type I, in *2% Acetic acid solution*.

**Collagen calibration standards:** Cut polyglactin mesh<sup>17</sup> into 17 11-mm  $\times$  11-mm squares, and place one square into 17 individual wells of a 24-well plate. Each well of the 24-well plate has a surface area of 220 mm<sup>2</sup> and a volume of 3.5 mL. In quadruplicate, prepare wells containing 0.050, 0.100, 0.200, and 0.400 mg of collagen by adding 25, 50, 100, and 200  $\mu$ L, respectively, of the *Collagen standard stock solution*. The remaining well to which no *Collagen standard stock solution* has been added is used as the blank. Allow the wells to air-dry.

**Sirius red solution:** 1 mg/mL of Direct Red 80 in saturated picric acid.

**1% (p-tert-Octylphenoxy)polyethoxyethanol solution:** Mix 10 mL of (p-tert-Octylphenoxy)polyethoxyethanol in 990 mL of *DPBS with Ca<sup>++</sup>, Mg<sup>++</sup> solution*.

<sup>16</sup> A suitable *Proteinase K solution* can be obtained from Roche Diagnostics Corp., Roche Applied Sciences, P.O. Box 50414, 9115 Hague Rd., Indianapolis, IN 46250-0414.

<sup>17</sup> A suitable polyglactin mesh can be obtained from Ethicon Co., Johnson & Johnson Corp., 425 Hoes Ln., P.O. Box 6800, Piscataway, NJ 08855.



**Analysis:** Thaw Construct Human Fibroblasts in Polyglactin Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to between 34° and 37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Polyglactin Scaffold unit. Cut three 11-mm × 11-mm sections of Construct Human Fibroblasts in Polyglactin Scaffold. Place each test section into separate wells of a 24-well plate. Add 200 µL of 1% (*p*-tert-Octylphenoxy)polyethoxyethanol solution to each sample. Shake on a rotating platform shaker at 100–150 rpm for 60–70 min at room temperature. Aspirate off the 1% (*p*-tert-Octylphenoxy)polyethoxyethanol solution, and rinse three times with 2 mL of DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution. Transfer each of the collagen standards to individual wells of the 24-well plate. Add 0.5 mL of *Sirius red* solution to each test sample and collagen standards. Shake on a rotating platform shaker at 100–150 rpm for 60 min at room temperature. Aspirate off the *Sirius red* solution from each well. Rinse each well twice with 2 mL of DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution. Add an additional 2 mL of DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution to each well, and allow to stand for 2 min. Aspirate off the DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution, and rinse twice more with 2 mL of DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution. Aspirate off all traces of DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution. Add 0.5 mL of Collagenase extraction solution to each well containing the Collagen calibration standards. Add 2.0 mL of Collagenase extraction solution to each well containing test samples. Rotate the plate on an orbital rotator at 150 rpm for 90 min at 37°. Transfer 200 µL from each well, to a suitable 96-well, flat-bottom plate. Read the absorbance of each aliquot at 540 nm. Dilute the Construct Human Fibroblasts in Polyglactin Scaffold sample preparation further with DPBS without Ca<sup>++</sup>, Mg<sup>++</sup> solution if the absorbance is greater than the absorbance of the highest of the Collagen calibration standards. Plot the responses of the Collagen calibration standards versus the amount, in mg of collagen, and calculate the regression line using the least-squares method. Determine the mg of collagen/Construct Human Fibroblasts in Polyglactin Scaffold section from the regression line and using the following equation:

$$\text{Result} = D \times A \times SC_{SR}$$

- D* = dilution factor (normally 4, unless the sample had to be further diluted)  
*A* = absorbance at 540 nm  
*SC<sub>SR</sub>* = slope of the regression line of the standards calculated above

**System suitability requirements:** The test is considered valid if the slope of the regression line is between 3.00 and 5.00 and the square of the correlation coefficient is greater than or equal to 0.950.

**Acceptance criteria:** The amount of collagen in individual Construct Human Fibroblasts in Polyglactin Scaffold 11-mm × 11-mm samples is 0.40–2.0 mg.

#### • **STERILITY TESTS (71)**

**Sample solution:** Thaw Construct Human Fibroblasts in Polyglactin Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to a temperature of 34°–37° for 2–3 min. The minimum amount of water in the water bath is 2 L/Construct Human Fibroblasts in Polyglactin Scaffold unit.

**Analysis:** Perform the test on 20 mL of the cryopreservative.

**Acceptance criteria:** Meets the requirements

#### • **BACTERIAL ENDOTOXINS TEST (85)**

**Sample:** Thaw Construct Human Fibroblasts in Polyglactin Scaffold by placing the tissue, still in its ethyl vinyl acetate bag, in a water bath heated to a temperature of 34°–37° for 2–3 min. The minimum amount of water in

the water bath is 2 L/Construct Human Fibroblasts in Polyglactin Scaffold unit. Remove the unit from the ethyl vinyl acetate bag, and immerse in 25 mL of LAL Reagent Water.

**Analysis:** Extract for 60 min at 37° with shaking on an orbital shaker set at 125 revolutions/min. Remove a 4-mL aliquot of the extract for testing.

**Acceptance criteria:** It contains NMT 0.5 USP Endotoxin Unit/mL.

#### **ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Construct Human Fibroblasts in Polyglactin Scaffold is aseptically packaged and supplied frozen in a clear ethyl vinyl acetate bag. The solution within the bag is a saline-based cryoprotectant supplemented with 10% dimethyl sulfoxide and bovine serum to facilitate long-term storage. An aluminum-plastic foil envelope surrounds the bag for its protection. Construct Human Fibroblasts in Polyglactin Scaffold should be stored at  $-75 \pm 10^\circ$  for no longer than 6 months.
- **LABELING:** The label indicates the dimensions of the Construct Human Fibroblasts in Polyglactin Scaffold material enclosed. It contains the expiry date, the required storage conditions, and the lot number. The label cautions that Construct Human Fibroblasts in Polyglactin Scaffold is not to be used if the package shows signs of damage. Additional labeling requirements include instructions on the proper thawing and handling of Construct Human Fibroblasts in Polyglactin Scaffold, the time frame for use after package opening, and a statement that cytotoxic agents are not to be used.
- **USP REFERENCE STANDARDS, Authentic Visual References (11)**  
 USP Construct Human Fibroblasts in Polyglactin Scaffold Reference Photomicrographs  
 [NOTE—These three photomicrographs represent examples of passing units. They are specified to assist in ascertaining histological quality.]
- **USP REFERENCE STANDARDS (11)**  
 USP Endotoxin RS

### **Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold**

(Monograph title change—to become official December 1, 2015)

#### **DEFINITION**

Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold is a living, bilayered skin substitute derived from neonatal foreskins manufactured under Class 100 sterile conditions. The upper, epidermal layer is formed by human keratinocytes and has a well-differentiated stratum corneum. The inner, dermal layer is composed of human fibroblasts in a bovine Type I collagen lattice. Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold does not contain Langerhans cells, melanocytes, macrophages, lymphocytes, blood vessels, hair follicles, or any other epidermally derived components. The fibroblast and keratinocyte cell banks from which Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold is derived test negative for human and animal viruses, retroviruses, bacteria, fungi, yeast, mycoplasma, and tumorigenicity. The cell banks are also tested for normal human karyology and isoenzymes. The final product is tested for morphology, cell viability, and physical container integrity. Used tissue culture media are tested for mycoplasma and sterility. All materials derived from bovine sources originate from countries free of bovine spongiform encephalopathy.



## SPECIFIC TESTS

## • HISTOLOGICAL CHARACTERIZATION

**2.0 M Monobasic potassium phosphate:** Dissolve 13.61 g of anhydrous monobasic potassium phosphate in 50 mL of water.

**2.0 M Dibasic potassium phosphate:** Dissolve 17.42 g of anhydrous dibasic potassium phosphate in 50 mL of water.

**Phosphate-buffered saline solution (pH 7.1–7.5):** Combine 3.6 mL of 2.0 M Monobasic potassium phosphate, 16.4 mL of 2.0 M Dibasic potassium phosphate, 8 g of sodium chloride, and 1 L of water. Mix thoroughly.

**0.3% Acid alcohol:** To 100 mL of 70% alcohol, add 0.3 mL of hydrochloric acid and mix.

**Hematoxylin–alcohol solution:** Dissolve 2.5 g of hematoxylin in 25.0 mL of dehydrated alcohol, with heating.

**Potassium alum solution:** Dissolve 50.0 g of potassium alum in 500 mL of water, with heating.

**Hematoxylin staining solution:** Mix the Hematoxylin–alcohol solution and the Potassium alum solution, and heat to boiling as rapidly as possible with constant stirring. Do not heat for more than 1 min. Slowly add 0.185 g of sodium iodate, and reheat to a simmer until the solution becomes a deep purple. Remove from the heat, and quickly cool. Filter daily before use.

**Bluing agent:** Dissolve 200 mg of sodium bicarbonate and 40 mg of lithium carbonate in 63 mL of water and 37 mL of methanol and mix.

**Eosin solution:** Dissolve 1 g of eosin Y in 100 mL of alcohol. Filter daily before use.

**Analysis:** Remove three 2-cm diameter circular tissues from every 30-cm<sup>2</sup> section of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold (NLT 30% of the total unit area), using the appropriate size biopsy punch. Cut with a circular rocking motion to prevent crushing the tissue. Immerse the sections in 3.7% dimethoxymethane for 30 min, using a gentle rocking motion. Remove the sections, and lay on a cutting surface, dermal side (glossy side) down. Cut an approximately 3-mm-wide strip through the center of the specimen, using a new, single-edged razor blade. Place the strips in a histological microwave cassette, using suitable biopsy pads premoistened with Phosphate-buffered saline solution (pH 7.1–7.5) to hold the strips in place. Insert the cassette into a histological microwave processing rack, place the rack inside a suitable microwave container, and add sufficient Phosphate-buffered saline solution (pH 7.1–7.5) to completely cover the rack. Place the container in a microwave oven suitable for histological work,<sup>1</sup> and heat for 4 min at 55°.

Remove the Phosphate-buffered saline solution (pH 7.1–7.5), and add enough dehydrated alcohol to completely cover the rack. Return the container to the microwave oven, and heat for 4 min at 67°. Remove the alcohol, and add enough dehydrated isopropyl alcohol to completely cover the rack. Return the container to the microwave oven, and heat for 4 min at 74°.

Remove the isopropyl alcohol, and add enough suitable grade paraffin<sup>2</sup> that has been melted and held at 84° prior to use, to completely cover the rack. Return the container to the microwave oven, and heat for 7 min at 84°. Remove the histological microwave cassette from the container and rack while the paraffin is still melted, and disassemble, discarding the biopsy pads. Fill preheated embedding molds with molten paraffin<sup>3</sup> heated to 60°, and place on top of a preheated warm-

ing platform that is designed for histological work. Using forceps, remove the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold specimens from the cassette, and place the specimens in individual molds. Orient the specimens in the molds to enable cutting of a cross-or longitudinal section. Cool the paraffin by sliding the mold down the platform to its cool side until the paraffin has solidified. Maintain the specimen orientation with forceps during cooling, removing the forceps when the paraffin becomes translucent. Slide the paraffin block onto a histological cold plate to rapidly cool the block. Trim the paraffin block with a new single-edged razor blade to form a rectangle or slight trapezoid to within 5 mm of the tissue mass, if necessary. Cool the block at 4° for 15 to 30 min, and clamp the paraffin block into the block holder of the microtome. Fill a histological tissue-flotation water bath with fresh water, add an appropriate amount of a suitable histological adhesive,<sup>4</sup> and heat to a temperature 5° lower than the melting point of the paraffin. Properly mount the paraffin block into a microtome, adjusting as necessary. Set the microtome to make 5-μm thick cuts with a blade angle of 5 ± 2°. Insert into the knife holder a sharp stainless steel microtome knife that has been properly honed or a new disposable microtome knife, and cut a ribbon that contains 6–10 sections of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold. Pick up the ribbon with forceps, and stretch it across the tissue-flotation water bath. Separate 2 to 3 adjacent sections from the ribbon on the water bath. The selected sections should not be compressed, wrinkled, or scratched. Pick up the selected sections by dipping a microscope slide into the water bath under the floating sections, and gently lift the slide out of the water. Allow the mounted sections to air-dry completely, or dry the slide in a 60° oven for 1 h. The microscope slide with affixed tissue is sequentially immersed in 3 changes of a suitable histological, aliphatic xylene substitute,<sup>5</sup> 5 min/step, followed by two changes of dehydrated alcohol, 3 min/step. Sequentially immerse the slide in alcohol (for 3 min), running water rinse (3 min), Hematoxylin staining solution (6 min), running water rinse (7 min), 0.3% Acid alcohol (6 s), running water rinse (5 min), Bluing agent (1 s), running water rinse (5 min), Eosin solution (2 min), two changes of alcohol (3 min each step), four changes of dehydrated alcohol (3 min each step), and four changes of a suitable histological xylene substitute (3 min each step). Adjust the above immersion times as needed to suitably stain the tissue. Remove the slide from the last histological xylene substitute wash, and blot dry the back of the slide. Do not allow the tissue to dry. Affix a coverslip over the tissue, using a suitable coverslip mountant.

**Microscopic specifications:** A light microscope with 4×, 10×, 20×, and 40× objectives installed in a revolving nosepiece; a 10× widefield ocular with 10 to 19 mm per 100 microdisk reticle installed; and a 10× widefield ocular with grid reticle installed.

**Microscopic and morphological characteristics:**

Score the three Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold sections for epidermal and dermal aspects, using the light microscope. Evaluate the slides from each of the sections taken. Average the aspect values for each section ( $n = 3$ ) to determine the overall aspect score for the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold unit. When examined microscopically, Construct Human Keratinocytes and Fibroblasts

<sup>1</sup> A microwave oven suitable for histological preparation can be obtained from Energy Beam Sciences, Inc., 11 Bowles Road, P.O. Box 14508, Agawam, MA or equivalent.

<sup>2</sup> A suitable paraffin for use is Accumate™ Tissue Embedding/Infiltration Medium, which can be obtained from Sigma Diagnostics, P.O. Box 468, St. Louis, MO 63178 or equivalent.

<sup>3</sup> A suitable paraffin for use is Paraplast® X-Tra Tissue Embedding Medium ASTM, melting point 50–54°, which can be obtained from Fisher Scientific, 300 Industry Drive, Pittsburgh, PA 15275 or equivalent.

<sup>4</sup> A suitable histological adhesive for use is Histoslide® Adhesive, which can be obtained from Poly Scientific R & D Corp., 70 Cleveland Ave., Bay Shore, NY 11706-1282 or equivalent.

<sup>5</sup> A suitable histological xylene substitute is Shandon Xylene Substitute from Thermo Scientific (<http://www.thermoscientific.com>) or equivalent.



in Bovine Collagen Scaffold shows a bilayered construct resembling the epidermal and dermal layers of human skin. Using USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrographs of passing and failing articles for comparison, Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold meets the requirements for epidermal aspects, including epidermal coverage, epidermal development, and keratinocyte aspect, and meets the requirements for dermal aspects, including dermal matrix thickness, fibroblast density, and matrix aspect, as described below.

#### Epidermal aspects

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 1 for an example of a passing unit.]

**Acceptance criteria for Epidermal coverage:** NLT 95% of the dermal matrix present on the slide is covered with epidermal keratinocytes.

**Acceptance criteria for Epidermal development:** NLT 70% of the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold epithelium is composed of three distinct cell layers.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 2 for an example of a failing unit.]

The basal cell layer of the epithelium is at least 1 cell thick, consisting of keratinocytes with a cuboidal-columnar shape.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 3 for an example of a failing unit.]

The suprabasal layer is composed of stratified cells and is at least 5 cells thick. Suprabasal cells closest to the basal layer are cuboidal in shape; cells become progressively stratified the closer they are to the uppermost, squamous cell layer. The squamous cell layer on the apical surface is cornified and at least 1 cell thick.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 4 for an example of a failing unit.]

The uppermost cell layer of the epithelium is analogous to the stratum corneum of human skin and is composed of one or more rows of flat, scaly cells that are nonliving and keratinized.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 5 for an example of a failing unit.]

**Acceptance criteria for Keratinocyte aspects:** NLT 95% of the basal keratinocytes have basophilic cytoplasm that neither has distinct vacuoles nor is necrotic. See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 6 for an example of a failing unit. NLT 80% of suprabasal cells (excluding those in the upper 20% of the cell layer closest to the squamous layer) have basophilic cytoplasm. Furthermore, these basophilic suprabasal cells do not have distinct vacuoles and are neither necrotic nor keratinized.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrographs 7 and 8 for examples of failing units.]

#### Dermal aspects

Five randomly selected fields/slide will be evaluated for dermal matrix thickness and fibroblast density. The five fields will be averaged to obtain the final value for each section.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrograph 1 for an example of a passing unit.]

#### Acceptance criteria for Dermal aspects

**Dermal matrix thickness:** The Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold

dermal layer is NLT 40  $\mu$ m thick and is composed of several rows of flat dermal cells.

**Fibroblast density:** The dermal matrix contains an average of at least four nonpyknotic nuclei present per microscopic field (field = 20 grid squares of reticle when using the 10 $\times$  widefield ocular and 40 $\times$  objective).

**Matrix aspect:** NLT 95% of the dermal matrix collagen stains uniformly with no large holes or inclusions present.

[NOTE—See USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrographs 9 and 10 for examples of failing units.]

#### • GENE EXPRESSION PROFILE

**RNA extraction solution:** Use an aqueous phenol and guanidine isothiocyanate solution suitable for RNA extraction.<sup>6</sup>

**DEPC-treated water:** Add 0.2 mL of diethylpyrocarbonate (DEPC) to 100 mL of sterile Purified Water, shake vigorously, and allow to stand for at least 12 h. Autoclave the resulting solution for 15 min, using the liquid cycle, to inactivate residual DEPC. Prepare fresh as needed.

**5X Reaction buffer:** Prepare a solution of 375 mM potassium chloride, 15 mM magnesium chloride, and 250 mM tris(hydroxymethyl) aminomethane hydrochloride. Adjust to a pH of 8.3.

**10X Reaction buffer:** Prepare a solution of 500 mM potassium chloride and 100 mM tris(hydroxymethyl) aminomethane hydrochloride. Adjust to a pH of 8.3.

**Oligo-deoxythymidine solution:** Prepare a 20-mM oligo-deoxythymidine (primer length: 18) solution, using a suitable buffer.<sup>7</sup>

**dNTP solution I:** Using a suitable buffer,<sup>7</sup> prepare a solution of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate in which the concentration of each component is 10 mM.

**dNTP solution II:** Prepare a solution, in water, of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate, in which the concentration of each component is 10 mM.

**Ribonuclease inhibitor solution:** Prepare a solution containing 40 units of ribonuclease inhibitor/mL of a suitable buffer.<sup>7</sup>

**Reverse transcriptase buffer:** Prepare a solution of 0.1 M sodium chloride, 0.1 mM edetate disodium, 1.0 mM dithiothreitol, 0.01% nonylphenol polyoxyethylene ether, 50% glycerin, and 200 mM tris(hydroxymethyl) aminomethane hydrochloride. Adjust to a pH of 7.5.

**Reverse transcriptase solution:** Prepare a solution containing 200 units of Moloney-Murine Leukemia Virus reverse transcriptase/ $\mu$ L in Reverse transcriptase solution.

**DNA primer pairs:** Prepare individual 20- $\mu$ M solutions of the following DNA primer pairs, using deoxyribonuclease- and ribonuclease-free water.

Table 1

Transforming growth factor $\beta$ 1 (TGF $\beta$ 1):	
TGF $\beta$ 1-3'	agg ctc caa atg tag ggg cag g
TGF $\beta$ 1-5'	gcc ctg gac acc aac tat tgc t
Interleukin-1 $\alpha$ (IL1 $\alpha$ ):	
IL1 $\alpha$ -3'	tag tgc cgt gag ttt ccc aga aga aga gga gg

<sup>6</sup> A suitable RNA extraction solution is Trizol® reagent, which can be obtained from Life Technologies, (<https://www.lifetechnologies.com>) or equivalent.

<sup>7</sup> A suitable buffer can be obtained from the RT-for-PCR Kit, Clontech, 1290 Terra Bella Ave., Mountain View, CA 94043 or equivalent.



Table 1 (Continued)

Transforming growth factor $\beta$ 1 (TGF $\beta$ 1):	
IL1 $\alpha$ -5'	caa gga gag cat ggt ggt agt agc aac caa cg
Interleukin-4 (IL4):	
IL4-3'	acg tac tct ggt tgg ctt cct tca cag gac ag
IL4-5'	cgg caa ctt tga cca cgg aca caa gtg cga ta
Platelet-derived growth factor A:	
PDGF-A-3'	ctg ctt cac cga gtg cta caa tac ttg ct
PDGF-A-5'	aga agt cca ggt gag gtt aga gga qcat
Glyceraldehyde-3-phosphate dehydrogenase:	
G3PDH-3'	cat gtg ggc cat gaq gtc cac cac
G3PDH-5'	tga agc tcg gag tca acg gat ttg qt

**DNA polymerase solution:** Prepare a solution containing five units of deoxyribonucleic acid polymerase/mL of a solution of 100 mM potassium chloride, 0.1 mM edetate disodium, 1 mM dithiothreitol, 0.5% polyoxyethylene (20) sorbitan monolaurate, 0.5% nonylphenol polyoxyethylene ether, 50% glycerol, and 20 mM tris(hydroxymethyl) aminomethane hydrochloride. Adjust to a pH of 8.0.

#### Analysis

**RNA extraction:** Remove three 2-cm diameter circular sections from every 30 cm<sup>2</sup> of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold (NLT 30% of the total unit area), using the appropriate size biopsy punch. Transfer each piece of tissue to individual polypropylene microcentrifuge tubes. Add 1.0 mL of RNA extraction solution to each tube, homogenize by repetitive pipetting, and incubate the samples for 5 min at room temperature. To each tube, add 0.2 mL of chloroform, mix on a vortex mixer, and centrifuge at 12,000  $\times$  g for 1.5 min at 2°–8°. Transfer the upper, aqueous phase to a second tube, add 0.5 mL of isopropanol, and incubate for 30 min to overnight at –20°. Centrifuge at 12,000  $\times$  g for 15 min, discard the supernatants by aspiration, and add 75% alcohol to each pellet. Mix the sample on a vortex mixer, centrifuge at 12,000  $\times$  g for 2 min, and discard the supernatants by aspiration without disturbing the RNA pellets. Recentrifuge at 12,000  $\times$  g for 2 min, and remove the remaining supernatants with a small-volume (20  $\mu$ L or smaller capacity) micropipet. Air-dry the pellets for 5 min at room temperature by keeping the microcentrifuge cap off, and resuspend each pellet in 50  $\mu$ L of DEPC-treated water. Bring absorbance into linear range by diluting 5  $\mu$ L of each suspension with 195  $\mu$ L of DEPC-treated water. Transfer the samples to suitable quartz microplates or cuvettes and determine the absorbance of the RNA solution at wavelengths of 260 and 280 nm, using a spectrophotometer and DEPC-treated water as the blank. The ratio of the absorbance at 260 versus 280 nm should be NLT 1.65. If this ratio is less than 1.65, mix the resuspended pellet by repetitive pipetting, and repeat the dilution step and absorbance measurement. If this fails to raise the absorbance ratio, repeat the RNA extraction for that sample by adding 1 mL of RNA extraction solution, and proceed as above, beginning with "incubate the sample for 5 min at room temperature".

Determine the concentration of RNA, in  $\mu$ g/mL:

$$\text{Result} = F \times A \times D$$

F = conversion factor, 40  
A = absorbance at 260 nm  
D = dilution factor

Adjust the volume of the RNA solutions with additional DEPC-treated water to bring the concentration of RNA to about 80  $\mu$ g/mL. If the absorbance at 260 nm is less than 0.05, discard the sample, and repeat the RNA extraction on a fresh sample.

**Synthesis of cDNA:** To separate, individual thin-walled polymerase chain reaction (PCR) tubes, add 12.5  $\mu$ L of the RNA solution from samples 1, 2, and 3 (3 reaction tubes total). Add 1  $\mu$ L of Oligo-deoxythymidine solution to each tube, and incubate at 72° for 2 min to anneal the oligo-deoxythymidine to the mRNA. Place the tubes in an ice bath, and to each tube, add 4  $\mu$ L of 5X Reaction buffer, 1  $\mu$ L of dNTP solution I, 0.5  $\mu$ L of Ribonuclease inhibitor solution, and 1  $\mu$ L of Reverse transcriptase solution. Incubate at 42° for 1 h to synthesize cDNA, and then incubate at 94° for 5 min to inactivate the reverse transcriptase. To each tube, add 80  $\mu$ L of DEPC-treated water and mix.

#### cDNA positive control<sup>a</sup>

**Polymerase chain reaction amplification of cDNA:** For each of the five DNA primer pairs, label five individual centrifuge tubes (5 tubes total). Add the following to each centrifuge tube: 135.8  $\mu$ L of DEPC-treated water; 10.5  $\mu$ L of dNTP solution II; 21  $\mu$ L of 10X Reaction buffer; 3.5  $\mu$ L of the appropriate 5' primer; 3.5  $\mu$ L of the appropriate 3' primer; and 12.6  $\mu$ L of 25 mM magnesium chloride. Close, mix on a vortex mixer, and pulse spin in a microcentrifuge. Add 2.1  $\mu$ L of DNA polymerase solution to each centrifuge tube, and mix by repetitive pipetting. For each primer pair, transfer 27  $\mu$ L of the resulting solution to five thin-walled PCR tubes. There should be a total of 25 PCR tubes. Add the following to the PCR tubes of each primer set:

Table 2

PCR tube number	Sample
1	3 $\mu$ L Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold sample 1 cDNA
2	3 $\mu$ L Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold sample 2 cDNA
3	3 $\mu$ L Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold sample 3 cDNA
4	3 $\mu$ L cDNA positive control
5 Negative control	3 $\mu$ L DEPC-treated water

Repeat for the remaining primer pairs. The positive control contains authentic cDNA of Transforming growth factor  $\beta$ , Interleukin-1 $\alpha$ , Interleukin-4, Platelet-derived growth factor A, and Glyceraldehyde-3-phosphate dehydrogenase, as appropriate for each primer set. Pulse spin the PCR tubes in a microcentrifuge to mix, and place the tubes in a single PCR thermal cycler. Cycling conditions are as follows.

Table 3

Melting temperature	94°
Melting time	45 s
Anneal temperature	58°
Anneal time	45 s
Elongation temperature	72°

<sup>a</sup> A suitable cDNA positive control can be obtained from Clontech, 1290 Terra Bella Ave., Mountain View, CA 94043.



Table 3 (Continued)

Elongation time	2 min
Number of cycles	30
Final elongation temperature	72°
Final elongation time	2 min

Terminate the PCR amplification by heating each tube to 72° for 7 min.

#### Electrophoresis identification

**Tris-boric acid buffer:** 89 mM of tris(hydroxymethyl)aminomethane, 89 mM of boric acid, and 2 mM of edetate disodium

**6X Loading buffer:** A solution containing 15% of a branched polymeric sucrose (400 kDa), 0.25% bromophenol blue, and 0.25% xylene cyanole FF

**Ethidium bromide solution:** 10 mg/mL Ethidium bromide in *Tris-boric acid buffer*

**Agarose gel:** A horizontal 2% agarose<sup>9</sup> gel in *Tris-boric acid buffer*

Once the gel is set, remove the comb, and place the gel into the electrophoresis chamber with the comb end of the gel situated closest to the cathode terminal. Fill the electrophoresis chamber with *Tris-boric acid buffer* until the buffer reaches 3–5 mm over the surface of the gel.

**100-bp DNA ladder markers:** A solution containing 10 DNA fragments covering the range of 100–1000 base pairs (bp) in 100-bp increments, with a total DNA content of approximately 100 ng/μL (15–20 ng of DNA per band) in an appropriate buffer<sup>10</sup>

**Analysis:** Dilute the 25 PCR samples prepared in the *Polymerase chain reaction amplification of cDNA* with *6X Loading buffer* so that the final concentration of the buffer is one-sixth of its original concentration. Load 5 μL of the *100-bp DNA ladder markers* in the first lane of the agarose gel. Load 10 μL of each PCR sample into each gel well, and attach the cathode to the terminal close to the loaded wells. Attach the anode to the terminal farthest from the loaded wells, and apply 120 V to the gel. Run the gel until the bromophenol blue is two-thirds the length of the gel. Remove the gel from the electrophoresis apparatus, and place it in a tray containing enough *Ethidium bromide solution* to cover the gel. Slowly agitate the gel on a shaker table for 30 min. Completely remove the *Ethidium bromide solution* from the tray, add an equal amount of *Tris-boric acid buffer*, and slowly agitate the gel on a shaker table for 60 min. Place the gel on a 312-nm UV light source, photograph the gel, and inspect the image for bands that have migrated from each individual well. If a band appears, it is verified for size in base pairs by comparing it to the lane for the *100-bp DNA ladder markers*.

**System suitability:** If a band appears and it is of the appropriate size, it is considered positive. The analysis is considered valid if the positive controls show the appropriately sized cDNA-PCR products, no PCR product bands appear in the negative controls, and all bands are observed to be visually discrete.

**Acceptance criteria:** The lanes of the agarose gel that correspond to Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold show cDNA bands for *Interleukin-1α* (expected PCR product band size of 491 base pairs, limit of detection NLT  $9.6 \times 10^{-21}$  moles); *Platelet-derived growth factor A* (expected PCR product band size of 304 base pairs, limit of detection NLT  $1.5 \times 10^{-20}$  moles); *Transforming growth factor β1*

(expected PCR product band size of 161 base pairs, limit of detection NLT  $1.5 \times 10^{-20}$  moles; and *Glyceraldehyde-3-phosphate dehydrogenase* (expected PCR product band size of 983 base pairs); but not *Interleukin-4* (expected PCR product band size of 344 base pairs, limit of detection NLT  $1.5 \times 10^{-22}$  moles). If one of the replicates tested yields results discordant with the other two replicates, repeat the test, and accept only if all three replicates are concordant.

#### • BARRIER INTEGRITY ASSESSMENT

**Ham's F-12 tissue culture medium:** Prepare a solution that contains the following:

Table 4

Component	mg/mL
L-Alanine	8.91
L-Arginine hydrochloride	210.7
L-Asparagine monohydrate	15.01
L-Aspartic acid	13.30
L-Cysteine hydrochloride monohydrate	35.12
L-Glutamic acid	14.70
L-Glutamine	146.2
Aminoacetic acid	7.51
L-Histidine hydrochloride monohydrate	20.96
L-Isoleucine	3.94
L-Leucine	13.12
L-Lysine hydrochloride	36.54
L-Methionine	4.48
L-Phenylalanine	4.96
L-Proline	34.53
L-Serine	10.51
L-Threonine	11.91
L-Tryptophan	2.04
L-Tyrosine disodium	6.71
L-Valine	11.71
Calcium chloride	44.00
Cupric sulfate, pentahydrate	0.0025
Ferric sulfate, heptahydrate	0.834
Potassium chloride	223.7
Magnesium chloride	57.22
Sodium chloride	7599.0
Sodium phosphate, dibasic	142.0
Zinc sulfate, heptahydrate	0.863
D-Biotin	0.0073
D-Calcium pantothenate	0.238
Choline chloride	13.96
Folic acid	1.30
Hypoxanthine	4.04
Inositol	18.02
Niacinamide	0.0366
Pyridoxine hydrochloride	0.0617
Riboflavin	0.0376
Thiamine hydrochloride	0.337
Thymidine	0.727
Cyanocobalamin	1.36
α-Lipoic acid	0.206
Linoleic acid	0.0841
Dextrose	1801.6
Phenol red, sodium	1.30
Sodium pyruvate	110.0
Putrescine dihydrochloride	0.161
Sodium bicarbonate	1176.0

<sup>9</sup> An agarose suitable for electrophoresis analysis of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold cytokine PCR product is SeaKem® GTG agarose and can be obtained from Lonza Inc., 90 Boroline Road, Allendale, NJ 07401 or equivalent.

<sup>10</sup> A suitable buffered solution of *100-bp DNA ladder markers* can be obtained from Lonza Inc., 90 Boroline Road, Allendale, NJ 07401 or equivalent.



**Tritiated water:** 2.0  $\mu\text{Ci/mL}$

**Percutaneous absorption apparatus:** Prepare the apparatus as described below.<sup>11</sup>

**Six-well cell culture plate:** The dimensions are inner diameter, 35 mm; depth, 18 mm.

**Cell culture well insert:** Each well is a plastic cylinder with inner length, 15 mm; inner diameter, 24 mm; outer diameter, 27 mm, with a flanged end extending 4 mm from the outer diameter. The inner diameter opposite the flanged end is covered by a taut polycarbonate membrane having a porosity of 3  $\mu\text{m}$ . The flange should allow the *Cell culture well insert* to be suspended in the well of a *Six-well cell culture plate*, leaving a 3-mm space between the bottom of the *Cell culture well insert* and the inner bottom surface of the *Six-well cell culture plate*.

**Percutaneous absorption insert:** Use a polytetrafluoroethylene cylinder having the following dimensions: length, 20 mm; inner diameter, 20 mm; outer diameter, 23 mm with a flanged end extending 3 mm from the outer diameter. Ten mm from the flanged end of the cylinder, the inner diameter begins to funnel so that the inner diameter at 10 mm from the flanged end is 20 mm, and the inner diameter at 15 mm from the flanged end is 8 mm. From 15 to 20 mm from the flanged end, the inner diameter remains at 8 mm. The outer diameter of the cylinder remains constant at 23 mm. The flanged end is considered to be the top of the component.

**Silicon grease:** Use high-vacuum silicon grease suitable for glass.<sup>12</sup>

**Analysis:** Fill each well of the *Six-well cell culture plate* with 1.5 mL of *Ham's F-12 tissue culture medium*. Remove two 2-cm circular sections from every 30 cm<sup>2</sup> of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold (NLT 20% of the total unit area), using the appropriate size biopsy punch. Transfer each excised section to a separate *Cell culture well insert*, dermal side down on the polycarbonate membrane. Using forceps, gently smooth out the section to remove any wrinkles. Apply a narrow ring of *Silicon grease* to the underside of the *Percutaneous absorption insert*, and place the insert into the *Cell culture well insert*, grease side down, onto the epidermal surface of the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold biopsy, with slight pressure to form a tight seal. Do not allow any grease to enter the 8-mm diameter exposed area of the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold surface. Place the *Cell culture well insert* containing the *Percutaneous absorption insert* into one of the wells of the *Six-well cell culture plate* containing 1.5 mL of *Ham's F-12 tissue culture medium*. Apply 1.0 mL of *Tritiated water* to the exposed surface of the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold unit in the *Percutaneous absorption insert*, and incubate at ambient temperature for 6 h. At the end of each h, transfer the *Cell culture well insert* containing the *Percutaneous absorption insert* to a new well within the *Six-well cell culture plate* containing 1.5 mL of fresh *Ham's F-12 tissue culture medium*. After the 6-h incubation, remove the *Cell culture well insert*. Remove a 0.5-mL aliquot of *Ham's F-12 tissue culture medium* from each well of the *Six-well cell culture plate*, and transfer into individual scintillation vials. Dispense 0.5 mL of *Tritiated water* to a separate scintillation vial as a control.

<sup>11</sup> A suitable *Percutaneous absorption apparatus*, not including the *Percutaneous absorption insert*, is a Costar® 6-well culture cluster, flat bottom with lid, and a Costar® Transwell®, 24 mm in a 6-well cluster plate with lid and can be obtained from Corning Life Sciences, 836 North Street, Building 300, Suite 3401, Tewksbury, MA 01876 or equivalent.

<sup>12</sup> A suitable *Silicon grease* is High Vacuum Silicon Lubricant for Glass and can be obtained from Dow Corning Corporation, P.O. Box 0994, Midland, MI 48686-0994.

Add 4.5 mL of a suitable scintillation cocktail<sup>13</sup> to each scintillation vial and gently mix. Place the scintillation vials into a liquid scintillation counter, and count the emissions in the tritium spectrum for 60 s. Average the counts for each of the six time points (punch average) and duplicate sections (unit average). Determine the percent penetration per h:

$$\text{Result} = F \times (C_s/C_c)$$

$F$  = conversion factor, 150

$C_s$  = counts/min of the 0.5-mL aliquot of the *Ham's F-12 tissue culture medium* taken at the end of the incubation period

$C_c$  = counts/min in the 0.5-mL aliquot of *Tritiated water*

**Acceptance criteria:** NMT 1.97% penetration is found.

#### • METABOLIC ACTIVITY ASSESSMENT

**Dulbecco's modified Eagle's tissue culture medium:**

Prepare a solution that contains the following:

Table 5

Component	mg/L
Calcium chloride	264.9
Ferric nitrate, nonahydrate	0.10
Potassium chloride	400.0
Magnesium sulfate, heptahydrate	200.0
Sodium chloride	6,400.0
Sodium bicarbonate	3,700.0
Sodium phosphate, monobasic (monohydrate)	125.0
Dextrose	4,500.0
Phenol red	15.0
Sodium pyruvate	110.0
L-Arginine hydrochloride	84.0
L-Cystine	48.0
Aminoacetic acid	30.0
L-Histidine hydrochloride monohydrate	42.0
L-Isoleucine	104.8
L-Leucine	104.8
L-Lysine hydrochloride	146.2
L-Methionine	30.0
L-Phenylalanine	66.0
L-Serine	42.0
L-Threonine	95.2
L-Tryptophan	16.0
L-Tyrosine	72.0
L-Valine	93.6
D-Calcium pantothenate	4.0
Choline chloride	4.0
Folic acid	4.0
Inositol	7.0
Nicotinamide	4.0
Pyridoxine hydrochloride	4.0
Riboflavin	0.40
Thiamine hydrochloride	4.0

**MTT solution:** Dissolve 0.33 g of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide in 1 L of *Dulbecco's modified Eagle's tissue culture medium*, with constant stirring. Pass the solution through a suitable size filter having a 0.2- $\mu\text{m}$  porosity.

**0.04 N Acidified isopropyl alcohol:** Add 3.45 mL of hydrochloric acid to 1 L of isopropyl alcohol, and mix thoroughly. Store at room temperature no longer than 6 months.

**Analysis:** Immerse the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold in separate

<sup>13</sup> A suitable scintillation cocktail is OptiPhase Supermix Perkin-Elmer Life Sciences, Inc., 549 Albany St., Boston, MA 02118 or equivalent.



40.0-mL portions of *MTT solution*, making sure that about 20 mL of *MTT solution* is under the sample article, and 20 mL of *MTT solution* is on the surface. Take care not to produce any bubbles. Incubate for 3 h at 37°, in an environment of air enriched with 10% carbon dioxide. After incubation, remove from the 37°, 10% carbon dioxide-enriched air environment. Transfer the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold to a suitable cutting surface, and, using an appropriate biopsy punch, remove three 8-mm diameter circular sections from every 30 cm<sup>2</sup> of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold (5% of unit area). Transfer each punch to individual snap-top test tubes. Add 0.9 mL of 0.04 N Acidified isopropyl alcohol to each tube, making sure that the tissue is completely submerged. If not submerged, use forceps to place the sample into the 0.04 N Acidified isopropyl alcohol. Cap each tube tightly, place on an orbital shaker, and shake for 1 h at a moderate setting. After 1 h, remove the tubes from the orbital shaker, and mix each tube on a vortex mixer. Inspect the tubes to make sure that the tissue samples continue to be submerged. If not, use forceps or another device to resubmerge the tissues. Return the tubes to the orbital shaker, and continue to shake for an additional 1 h. Remove the tubes from the orbital shaker, mix the tubes on a vortex mixer, and transfer a 0.2-mL aliquot to a suitable 96-well flat-bottom plate. Read the absorbance of each sample at 570 nm, using 0.2 mL of 0.04 N Acidified isopropyl alcohol as the blank.

**Acceptance criteria:** The average absorbance value is NLT 0.237.

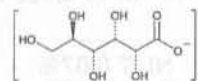
#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold is aseptically packaged in a Class 100 environment in single-use containers that preserve cell viability and product integrity. Store at controlled room temperature for NMT 5 days, and do not subject to freezing temperatures. The atmosphere within the package contains air enriched with 10% carbon dioxide. The device is translucent and off-white in color. The upper, epidermal surface is dull with small irregularities resulting from the cornification of keratinocytes, while the bottom surface is smooth and shiny in appearance. The device is packaged so that the dermal layer (glossy layer) is closest to the agarose-based nutrient medium. The packaging permits easy observation of the medium and provides ready access to the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold when needed. The medium contains all of the required nutrients for the living cell components of Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold plus an appropriate, nontoxic, pH-sensitive dye to indicate package breaches or microbial contamination. The medium should appear pink (pH 6.8–7.7) when compared to the enclosed pH color chart.
- **LABELING:** Label it to indicate the dimensions of the enclosed Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold, the expiry date, the required storage conditions, and the lot number. The label indicates that the enclosed Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold and surrounding medium are to be examined for signs of contamination or deterioration. The label also contains a pH color code to be used for determination of the acceptability of the pH of the Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold medium. The label cautions that Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold is not to be used if the package shows signs of damage or microbial contamination. Label it to indicate that sterile techniques are to be used in handling Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold and that

cytotoxic agents are not to be used. Label it to indicate the time frame for use after package opening.

- **USP REFERENCE STANDARDS, Authentic Visual References (11)** *USP Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold Reference Photomicrographs* [NOTE—These 10 photomicrographs represent examples of both passing and failing Construct Human Keratinocytes and Fibroblasts in Bovine Collagen Scaffold units. They are specified to assist in ascertaining histological quality.]

## Copper Gluconate



C<sub>12</sub>H<sub>22</sub>CuO<sub>14</sub> 453.84  
Copper, bis(D-gluconato-O<sup>1</sup>,O<sup>2</sup>)-;  
Copper D-gluconate (1:2) [527-09-3].

#### DEFINITION

Copper Gluconate contains NLT 98.0% and NMT 102.0% of copper gluconate (C<sub>12</sub>H<sub>22</sub>CuO<sub>14</sub>).

#### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Copper (191):** A 50-mg/mL solution meets the requirements.
- **B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST**  
Standard solution: 10 mg/mL of USP Potassium Gluconate RS  
Sample solution: 10 mg/mL of Copper Gluconate, heating in a water bath at 60°, if necessary, to dissolve  
Chromatographic system  
(See *Chromatography* (621), *Thin-Layer Chromatography*.)  
Mode: TLC  
Adsorbent: 0.25-mm layer of chromatographic silica gel  
Application volume: 5 µL  
Developing solvent system: Alcohol, ethyl acetate, ammonium hydroxide, and water (50:10:10:30)  
Spray reagent: Dissolve 2.5 g of ammonium molybdate in 50 mL of 2 N sulfuric acid in a 100-mL volumetric flask, add 1.0 g of ceric sulfate, swirl to dissolve, and dilute with 2 N sulfuric acid to volume.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Develop the chromatogram until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and dry at 110° for 20 min. Allow to cool, and spray with the *Spray reagent*. Heat the plate at 110° for about 10 min.  
**Acceptance criteria:** The principal spot of the *Sample solution* corresponds in color, size, and R<sub>f</sub> value to that of the *Standard solution*.

#### ASSAY

- **PROCEDURE**  
Sample: 1.5 g of Copper Gluconate  
Blank: 100 mL of water  
Titrimetric system  
(See *Titrimetry* (541).)  
Mode: Indirect titration  
Titrant: 0.1 N sodium thiosulfate VS  
Endpoint detection: Visual  
Analysis: Dissolve the *Sample* in 100 mL of water. Add 2 mL of glacial acetic acid and 5 g potassium iodide, mix, and titrate with *Titrant* to a light yellow color. Add 2 g of ammonium thiocyanate, and mix. Add 3 mL of



starch TS, and continue titrating to a milk-white endpoint. Perform the blank determination. Calculate the percentage of copper gluconate ( $C_{12}H_{22}CuO_{14}$ ) in the *Sample* taken:

$$\text{Result} = [(V_S - V_B) \times N \times F/W] \times 100$$

$V_S$  = Titrant volume consumed by the *Sample* (mL)  
 $V_B$  = Titrant volume consumed by the *Blank* (mL)  
 $N$  = actual normality of the *Titrant* (mEq/mL)  
 $F$  = equivalency factor, 453.8 mg/mEq  
 $W$  = *Sample* weight (mg)  
 Acceptance criteria: 98.0%–102.0%

#### IMPURITIES

##### • CHLORIDE AND SULFATE, Chloride (221)

Standard solution: 1.0 mL of 0.020 N hydrochloric acid

Sample: 1.0 g

Acceptance criteria: NMT 0.07%

##### • CHLORIDE AND SULFATE, Sulfate (221)

Standard solution: 1.0 mL of 0.020 N sulfuric acid

Sample: 2.0 g

Acceptance criteria: NMT 0.05%

##### • ARSENIC, Method I (211)

Test preparation: 1.0 g in 35 mL of water

Acceptance criteria: NMT 3 ppm

##### • LIMIT OF LEAD

[NOTE—For the preparation of all aqueous solutions and for the rinsing of glassware before use, use water that has been passed through a strong-acid, strong-base, mixed-bed ion-exchange resin. Select all reagents to have as low a content of lead as practicable, and store all reagent solutions in containers of borosilicate glass. Cleanse glassware before use by soaking in warm 8 N nitric acid for 30 min and by rinsing with deionized water.]

**Standard stock solution:** Transfer 10.0 mL of lead nitrate stock solution TS to a 100-mL volumetric flask. Add 40 mL of water and 5 mL of nitric acid, and dilute with water to volume.

**Standard solution:** Transfer 0.40 mL of *Standard stock solution* to a 100-mL volumetric flask. Add 50 mL of water and 1 mL of nitric acid, and dilute with water to volume. This solution contains 0.04 µg/mL of lead.

**Sample stock solution:** Transfer 4 g of Copper Gluconate to a 100-mL volumetric flask. Add 50 mL of water and 5 mL of nitric acid, and sonicate to dissolve the specimen. Dilute with water to volume. Transfer 4.0 mL of this solution to a second 100-mL volumetric flask. Add 50 mL of water and 1 mL of nitric acid, dilute with water to volume, and mix.

**Blank:** Transfer 1.2 mL of nitric acid to a 100-mL volumetric flask and dilute with water to volume.

**Sample solution A:** Mix 10.0 mL of the *Sample stock solution* with 10.0 mL of the *Blank*. This solution contains 0.00 µg/mL of added lead from the *Standard solution*.

**Sample solution B:** Mix 10.0 mL of the *Sample stock solution* with 4.0 mL of the *Standard solution* and 6.0 mL of *Blank*. This solution contains 0.008 µg/mL of added lead from the *Standard solution*.

**Sample solution C:** Mix 10.0 mL of the *Sample stock solution* with 7.0 mL of the *Standard solution* and 3.0 mL of *Blank*. This solution contains 0.014 µg/mL of added lead from the *Standard solution*.

**Sample solution D:** Mix 10.0 mL of the *Sample stock solution* with 10.0 mL of the *Standard solution*. This solution contains 0.020 µg/mL of added lead from the *Standard solution*.

#### Instrumental conditions

(See *Atomic Absorption Spectroscopy* (852).)

Mode: Graphite furnace atomic absorption spectrophotometry

Analytical wavelength: 283.3 nm

Lamp: Lead hollow-cathode

Argon flow rate: 3 L/min, or as noted

Graphite tube temperature: See Table 1.

Table 1

Temperature (°)	Time (s)
70	10
90	60
120	15
250 (no gas flow)	5
250	10
250 (no gas flow)	2
2000	3.2

Injection volume: 20 µL

#### Analysis

Samples: *Blank* and *Sample solutions A, B, C, and D*

The graphite tube is temperature-programmed to reach 2000° in about 2 min, as shown in Table 1. When the temperature reaches 2000°, determine the absorbance at 283.3 nm, corrected for background absorption. Inject the *Sample solutions* and *Blank*, and determine the absorbances. Correct the absorbance values from the *Sample solutions* by subtracting from each the absorbance value from the *Blank*. Plot the corrected absorbances of the *Sample solutions* versus their added lead concentrations, in µg/mL. Draw the straight line best fitting the four points, and extrapolate the line until it intercepts the concentration axis. From the intercept, determine the concentration,  $C$ , in µg/mL, of lead in *Sample solution A*.

Calculate the content of lead in the portion of Copper Gluconate taken:

$$\text{Result} = (C \times V)/W$$

$C$  = concentration of lead in the *Sample solution A* (µg/mL), determined from the intercept of the linear regression line

$V$  = volume of solvent taken to prepare the *Sample solution A* (mL)

$W$  = weight of Calcium Gluconate taken to prepare the *Sample solution A* (g)

Acceptance criteria: NMT 25 µg/g

##### • REDUCING SUBSTANCES

Sample: 1.0 g of Copper Gluconate

Blank: 10 mL of water

Titrimetric system

(See *Titrimetry* (541).)

Mode: Residual titration

Titrant: 0.1 N iodine VS

Back-titrant: 0.1 N sodium thiosulfate VS

Endpoint detection: Visual

**Analysis:** Transfer the *Sample* to a 250-mL conical flask, add 10 mL of water to dissolve the *Sample*, then add 25 mL of alkaline cupric citrate TS. Cover the flask, boil gently for 5 min, accurately timed, and cool rapidly to room temperature. Add 25 mL of 0.6 N acetic acid, 10.0 mL of *Titrant*, and 10 mL of 3 N hydrochloric acid, and titrate with *Back-titrant*, adding 3 mL of starch TS as the endpoint is approached. Perform the blank determination.

Calculate the percentage of reducing substances (as dextrose) in the *Sample* taken:

$$\text{Result} = [(V_B - V_S) \times N \times F/W] \times 100$$

$V_B$  = Back-titrant volume consumed by the *Blank* (mL)

$V_S$  = Back-titrant volume consumed by the *Sample* (mL)



$N$  = actual normality of the *Back-titrant* (mEq/mL)  
 $F$  = equivalency factor, 27 mg/mEq  
 $W$  = Sample weight (mg)  
 Acceptance criteria: NMT 1.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
 USP Potassium Gluconate RS

## Corticotropin Injection

### DEFINITION

Corticotropin Injection is a sterile solution, in a suitable diluent, of the material containing the polypeptide hormone having the property of increasing the rate of secretion of adrenal corticosteroids, which is obtained from the anterior lobe of the pituitary of mammals used for food by humans. Its potency is NLT 80.0% and NMT 125.0% of the potency stated on the label in USP Corticotropin Units. It may contain a suitable antimicrobial agent.

### IDENTIFICATION

#### • A. HPLC

**Solution A:** 0.1% Trifluoroacetic acid  
**Solution B:** 0.1% Trifluoroacetic acid in acetonitrile  
**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
1	75	25
17	70	30
27	70	30
27.5	20	80
32	20	80
32.5	75	25
35	75	25

**Standard solution:** 18.7 USP Corticotropin Units/mL of USP Corticotropin RS

**Sample solution:** 22 USP Corticotropin Units/mL of Corticotropin Injection

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** Fluorescence; excitation 295 nm, emission 355 nm

**Column:** 4.6-mm × 15-cm; 3-μm packing L1

**Temperatures**

**Sample tray:** 35°

**Column:** 35°

**Flow rate:** 1.0 mL/min

**Injection volume:** 25 μL

#### System suitability

**Sample:** Standard solution

#### Suitability requirements

**Relative standard deviation of the retention time:**  
 NMT 2%

#### Analysis

**Samples:** Standard solution and Sample solution

**Acceptance criteria:** The retention time of the corticotropin peak of the *Sample solution* corresponds to that of the *Standard solution*.

- **B.** Meets the requirements of the Assay

### ASSAY

#### Change to read:

#### • PROCEDURE

**Standard solution:** Pipet 2.5 mL of gelatin TS into an opened container of USP Corticotropin RS, and mix to obtain a solution with a concentration of 2.0 USP Corticotropin Units/mL. Using gelatin TS as a diluent, prepare three diluted *Standard solutions* such that the respective concentrations of corticotropin constitute a geometric series such as 1:2:4 or 1:3:9 and such that the quantity of corticotropin in each 0.5 mL lies within the range of 10–300 milliunits.

**Sample solution:** In the same manner, using the same diluent, dilute the Injection to give three *Sample solutions* corresponding in concentration to those of the *Standard solutions*.

**The animals:** Select healthy rats, of the same but either sex, that have been raised on a diet fully adequate with respect to vitamin and mineral content. Anesthetize the rats (IRA 1-Jul-2016) and remove the hypophysis from each by application of gentle suction through a fine-tipped tube. Between 16 and 48 h after the operation, select those rats weighing 80–180 g, but restrict the selection so that no rat is more than 30% heavier than the lightest, and the number of rats is an exact multiple of 6. Separate the selected rats into 6 groups, equal in size, of NLT 6 rats each, and assign at random one of the three diluted *Standard solutions* or one of the three *Sample solutions* to each group.

**Analysis:** Inject all rats of each group subcutaneously with the assigned test doses. Three h after the injection, anesthetize the rats, and remove both adrenal glands from each rat, free them from adhering tissue, and promptly weigh each pair on a suitable balance to the nearest 0.2 mg. Place the weighed glands from each rat in suitable vessels each containing 8.0 mL of metaphosphoric acid solution (1 in 40), and pulverize the glands by grinding with a small quantity of washed sand. Cover each vessel, and proceed similarly until all glands have been extracted.

**Ascorbic acid determination:** Filter the metaphosphoric acid extracts, and pipet 4 mL of each filtrate into suitable vessels each containing 4.0 mL of indophenol-acetate TS. Mix by shaking, and read the absorbance at 520 nm, with a suitable spectrophotometer. From the observed absorbance and the standard curve prepared as directed below, calculate the amount of ascorbic acid in mg/100 g of adrenal gland tissue. Prepare a standard concentration-absorbance curve, using three ascorbic acid solutions containing, respectively, 6.0, 8.0, and 10.0 μg/mL of USP Ascorbic Acid RS in metaphosphoric acid solution (1 in 40). Pipet into each of three suitable vessels, preferably spectrophotometer cells, 4 mL of indophenol-acetate TS. Add 4.0 mL of one of the three standard ascorbic acid solutions to one of the cells, mix, and promptly read the absorbance from the same instrument and under the same conditions as for the adrenal gland extracts. Repeat the process for the other two standard ascorbic acid solutions, plot the concentration-absorbance values, and draw the straight line best fitting the three plotted points.

**Calculation:** If there are no missing data; i.e., all groups of rats are the same size,  $f$ , then the following may be used. [NOTE—If there are missing values, then suitable software can be used and standard procedures followed for parallel line bioassays, including assessment of parallelism and linearity.] Tabulate the observed concentration of ascorbic acid in the adrenal glands of each rat, designated by the symbol  $y_{jkl}$ , where  $j = S$  (Standard) or  $U$  (Injection),  $k = 1, 2, \text{ or } 3$  for the three doses, and  $l =$



1, . . . ,  $f$  rats. Total the values of the  $y_{jkl}$ 's in each group as:

$$T_{jk} = \sum_{l=1}^f y_{jkl}$$

Then determine the following quantities:

$$T_a = \sum_{k=1}^3 (T_{0k} - T_{3k})$$

$$T_b = (T_{03} - T_{01}) + (T_{33} - T_{31})$$

$$V = (T_{03} - T_{01}) / (T_{33} - T_{31})$$

$$T_q = (T_{03} - 2T_{02} + T_{01}) + (T_{33} - 2T_{32} + T_{31})$$

$$T_{0q} = (T_{03} - 2T_{02} + T_{01}) - (T_{33} - 2T_{32} + T_{31})$$

$$s^2 = \frac{1}{n} \left[ \sum_{j,k,l} y_{jkl}^2 - \frac{1}{f} \sum_{j,k} T_{jk}^2 \right] \text{ where } n = 6(f-1)$$

$$\text{or } s^2 = \frac{1}{6} s_k^2 \text{ where } s_k^2 = \frac{1}{f-1} \sum_{l=1}^f (y_{jkl} - \frac{T_{jk}}{f})^2$$

$$F = \frac{T_q^2 + T_{0q}^2}{24fs^2}$$

If  $V \geq 0.75$  and  $V \leq 1.33$ , then the data satisfy parallelism. If  $F \leq F_{0.05, 2, n}$  where  $F_{0.05, 2, n}$  is the upper 0.05 percentage point of an  $F$  distribution with 2 and  $n$  degrees of freedom, then the data satisfy linearity. If both conditions are satisfied, determine the logarithm of potency of the Injection,  $M$ , taken as:

$$M = M' + \log R,$$

$$\text{where } M' = 4iT_a/(3T_b)$$

$i$  = interval between successive log doses of both the *Standard solution* and the *Sample solution*  
 $R$  =  $v_s/v_u$ , the ratio of the high dose of the *Standard solution* in USP Corticotropin Units ( $v_s$ ) to the high dose of the Injection in mL ( $v_u$ )

Determine the width,  $L$ , of the log confidence interval as:

$$L = 2\sqrt{(C-1)(CM')^2 + \frac{1}{3}i^2}$$

$$\text{where } C = \frac{T_a^2}{(T_b^2 - 4fs^2t_{0.025, n}^2)}$$

and  $t_{0.025, n}$  is the upper one-sided 0.025 percentage point (or two-sided 0.05 percentage point) of a  $t$ -distribution with  $n$  degrees of freedom.

**Replication:** Repeat the entire determination at least once. Test the agreement among the two or more independent determinations, and compute the weight for each (see *Design and Analysis of Biological Assays* (111), *Combination of Independent Assays*). Calculate the weighted mean log-potency  $\bar{M}$  and its confidence interval,  $L_c$  (see (111), *The Confidence Interval and Limits of Potency*). The potency,  $P$ , is satisfactory if  $P$  = antilog  $\bar{M}$  is 80%–125% of the labeled potency and if the confidence interval does not exceed 0.40.

**Acceptance criteria:** 80.0%–125.0%

## IMPURITIES

### • VASOPRESSIN ACTIVITY

**Solution A:** Dissolve 6.6 g of dibasic ammonium phosphate in about 950 mL of water, and adjust with concentrated phosphoric acid to a pH of 3.0. Dilute with water to 1 L.

**Mobile phase:** Acetonitrile and *Solution A* (13:87). Filter and degas.

[NOTE—The retention time of the vasopressin peak is very sensitive to small changes in the acetonitrile concentration.]

**Standard solution:** Dissolve the entire contents of a vial of USP Vasopressin RS in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 0.1 USP Vasopressin Units/mL.

**Sample solution:** Dissolve the entire contents of a vial of the Injection in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 2.0 USP Corticotropin Units/mL.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** About 1.5 mL/min

**Injection volume:** 20 μL

### System suitability

**Sample:** *Standard solution*

### Suitability requirements

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the vasopressin activity in USP Vasopressin Units/USP Corticotropin Unit:

$$\text{Result} = C_s \times [(r_u/r_s)/2]$$

$C_s$  = concentration of *Standard solution* (USP Vasopressin Units/mL)

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

**Acceptance criteria:** NMT 0.05 USP Vasopressin Units/USP Corticotropin Unit

## SPECIFIC TESTS

• **pH (791):** 3.0–7.0

• **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 3.1 USP Endotoxin Units/USP Corticotropin Unit

• **INJECTIONS AND IMPLANTED DRUG PRODUCTS (1):** Meets the requirements

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass. Store in a cold place.

• **LABELING:** If the labeling of Injection recommends intravenous administration, include specific information on dosage.

• **USP REFERENCE STANDARDS (11)**

USP Ascorbic Acid RS

USP Corticotropin RS

USP Endotoxin RS

USP Vasopressin RS

## Corticotropin for Injection

### DEFINITION

Corticotropin for Injection is the sterile, dry material containing the polypeptide hormone having the property of increasing the rate of secretion of adrenal corticosteroids, which is obtained from the anterior lobe of the pituitary of mammals used for food by humans. Its potency is NLT 80.0% and NMT 125.0% of the potency stated on the label in USP Corticotropin Units. It may contain a suitable antimicrobial agent and suitable diluents and buffers.



**IDENTIFICATION****• A. HPLC**

**Solution A:** 0.1% Trifluoroacetic acid

**Solution B:** 0.1% Trifluoroacetic acid in acetonitrile

**Mobile phase:** See Table 1.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	75	25
1	75	25
17	70	30
27	70	30
27.5	20	80
32	20	80
32.5	75	25
35	75	25

**Standard solution:** 18.7 USP Corticotropin Units/mL of USP Corticotropin RS

**Sample solution:** 22 USP Corticotropin Units/mL of Corticotropin for Injection

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Fluorescence; excitation 295 nm, emission 355 nm

**Column:** 4.6-mm × 15-cm; 3-μm packing L1

**Temperatures**

**Sample tray:** 35°

**Column:** 35°

**Flow rate:** 1.0 mL/min

**Injection volume:** 25 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation of the retention time:**  
NMT 2%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

**Acceptance criteria:** The retention time of the corticotropin peak of the *Sample solution* corresponds to that of the *Standard solution*.

**• B. Meets the requirements of the Assay****ASSAY**

**Change to read:**

**• PROCEDURE**

**Standard solution:** Pipet 2.5 mL of gelatin TS into an opened container of USP Corticotropin RS, and mix to obtain a solution with a concentration of 2.0 USP Corticotropin Units/mL. Using gelatin TS as a diluent, prepare three diluted *Standard solutions* such that the respective concentrations of corticotropin constitute a geometric series such as 1:2:4 or 1:3:9 and such that the quantity of corticotropin in each 0.5 mL lies within the range of 10–300 milliunits.

**Sample solutions:** Constitute the Corticotropin for Injection as directed in the labeling. In the same manner as the *Standard solutions* preparation, using the same diluent, dilute the reconstituted Corticotropin for Injection to give three *Sample solutions* corresponding in concentration to those of the *Standard solutions*.

**The animals:** Select healthy rats, of the same but either sex, that have been raised on a diet fully adequate with respect to vitamin and mineral content. Anesthetize the rats (see (IRA 1-jul-2016)) and remove the hypophysis from each by application of gentle suction through a fine-tipped tube. Between 16 and 48 h after the operation, select those rats weighing 80–180 g, but restrict the se-

lection so that no rat is more than 30% heavier than the lightest, and the number of rats is an exact multiple of 6. Separate the selected rats into 6 groups, equal in size, of NLT 6 rats each, and assign at random one of the three diluted *Standard solutions* or one of the three *Sample solutions* to each group.

**Analysis:** Inject all rats of each group subcutaneously with the assigned test doses. Three h after the injection, anesthetize the rats, and remove both adrenal glands from each rat, free them from adhering tissue, and promptly weigh each pair on a suitable balance to the nearest 0.2 mg. Place the weighed glands from each rat in suitable vessels each containing 8.0 mL of metaphosphoric acid solution (1 in 40), and pulverize the glands by grinding with a small quantity of washed sand. Cover each vessel, and proceed similarly until all glands have been extracted.

**Ascorbic acid determination:** Filter the metaphosphoric acid extracts, and pipet 4 mL of each filtrate into suitable vessels each containing 4.0 mL of indophenol-acetate TS. Mix by shaking, and read the absorbance at 520 nm, with a suitable spectrophotometer. From the observed absorbance and the standard curve prepared as directed below, calculate the amount of ascorbic acid in mg/100 g of adrenal gland tissue. Prepare a standard concentration-absorbance curve, using three ascorbic acid solutions containing, respectively, 6.0, 8.0, and 10.0 μg/mL of USP Ascorbic Acid RS in metaphosphoric acid solution (1 in 40). Pipet into each of three suitable vessels, preferably spectrophotometer cells, 4 mL of indophenol-acetate TS. Add 4.0 mL of one of the three standard ascorbic acid solutions to one of the cells, mix, and promptly read the absorbance from the same instrument and under the same conditions as for the adrenal gland extracts. Repeat the process for the other two standard ascorbic acid solutions, plot the concentration-absorbance values, and draw the straight line best fitting the three plotted points.

**Calculation:** If there are no missing data; i.e., all groups of rats are the same size,  $f$ , then the following may be used. [NOTE—If there are missing values, then suitable software can be used and standard procedures followed for parallel line bioassays, including assessment of parallelism and linearity.] Tabulate the observed concentration of ascorbic acid in the adrenal glands of each rat, designated by the symbol  $y_{jkl}$ , where  $j = S$  (Standard) or  $U$  (Injection),  $k = 1, 2$ , or  $3$  for the three doses, and  $l = 1, \dots, f$  rats. Total the values of the  $y_{jkl}$ 's in each group as:

$$T_{jk} = \sum_{l=1}^f y_{jkl}$$

Then determine the following quantities:

$$T_a = \sum_{k=1}^3 (T_{Uk} - T_{Sk})$$

$$T_b = (T_{U3} - T_{U1}) + (T_{S3} - T_{S1})$$

$$V = \frac{(T_{U3} - T_{U1})}{(T_{S3} - T_{S1})}$$

$$T_q = (T_{U3} - 2T_{U2} + T_{U1}) + (T_{S3} - 2T_{S2} + T_{S1})$$

$$T_{aq} = (T_{U3} - 2T_{U2} + T_{U1}) - (T_{S3} - 2T_{S2} + T_{S1})$$

$$s^2 = \frac{1}{n} \left[ \sum_{j,k,l} y_{jkl}^2 - \frac{1}{f} \sum_{j,k} T_{jk}^2 \right] \text{ where } n = 6(f-1)$$

$$\text{or } = \frac{1}{6} s_{jk}^2 \text{ where } s_{jk}^2 = \frac{1}{f-1} \sum_{l=1}^f (y_{jkl} - \frac{T_{jk}}{f})^2$$

$$F = \frac{T_a^2 + T_{aq}^2}{24fs^2}$$



If  $V \geq 0.75$  and  $V \leq 1.33$ , then the data satisfy parallelism. If  $F \leq F_{0.05,2,n}$ , where  $F_{0.05,2,n}$  is the upper 0.05 percentage point of an  $F$  distribution with 2 and  $n$  degrees of freedom, then the data satisfy linearity. If both conditions are satisfied, determine the logarithm of potency of the Injection,  $M$ , taken as:

$$M = M' + \log R,$$

$$\text{where } M' = 4i\bar{T}_d/(3T_b)$$

- $i$  = interval between successive log doses of both the *Standard solution* and the *Sample solution*  
 $R$  =  $v_s/v_u$ , the ratio of the high dose of the *Standard solution* in USP Corticotropin Units ( $v_s$ ) to the high dose of the Injection in mL ( $v_u$ )

Determine the width,  $L$ , of the log confidence interval as:

$$L = 2\sqrt{(C-1)(C(M')^2 + \frac{1}{3}i^2)}$$

$$\text{where } C = \frac{T_b^2}{(T_b^2 - 4fs^2t_{0.025,n}^2)}$$

and  $t_{0.025,n}$  is the upper one-sided 0.025 percentage point (or two-sided 0.05 percentage point) of a  $t$ -distribution with  $n$  degrees of freedom.

**Replication:** Repeat the entire determination at least once. Test the agreement among the two or more independent determinations, and compute the weight for each (see *Design and Analysis of Biological Assays* (111), *Combination of Independent Assays*). Calculate the weighted mean log-potency  $\bar{M}$  and its confidence interval,  $L_c$  (see (111), *The Confidence Interval and Limits of Potency*). The potency,  $P$ , is satisfactory if  $P = \text{antilog } \bar{M}$  is 80%–125% of the labeled potency and if the confidence interval does not exceed 0.40.

Acceptance criteria: 80.0%–125.0%

## PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

## IMPURITIES

### • VASOPRESSIN ACTIVITY

**Solution A:** Dissolve 6.6 g of dibasic ammonium phosphate in 950 mL of water, and adjust with concentrated phosphoric acid to a pH of 3.0. Dilute with water to 1 L.

**Mobile phase:** Acetonitrile and *Solution A* (13:87)

[NOTE—The retention time of the vasopressin peak is very sensitive to small changes in the acetonitrile concentration.]

**Standard solution:** Dissolve the entire contents of a vial of USP Vasopressin RS in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 0.1 USP Vasopressin Units/mL.

**Sample solution:** Dissolve the entire contents of a vial of Corticotropin for Injection in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 2.0 USP Corticotropin Units/mL.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

Flow rate: 1.5 mL/min

Injection volume: 20  $\mu$ L

### System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

### Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the vasopressin activity in USP Vasopressin

Units/USP Corticotropin Unit:

$$\text{Result} = C_s \times [(r_u/r_s)/2]$$

$C_s$  = concentration of the *Standard solution* (USP Vasopressin Units/mL)

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

Acceptance criteria: NMT 0.05 USP Vasopressin Units/USP Corticotropin Unit

## SPECIFIC TESTS

- **pH** (791): 2.5–6.0, in a solution constituted as directed in the labeling supplied by the manufacturer
- **PARTICULATE MATTER IN INJECTIONS** (788): Meets the requirements for small-volume injections
- **STERILITY TESTS** (71): Meets the requirements
- **BACTERIAL ENDOTOXINS TEST** (85): NMT 3.1 USP Endotoxin Units/USP Corticotropin Unit
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS** (1), *Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and clarity of solutions and LABELING* (7), *Labels and Labeling for Injectable Products*: Meets the requirements

## ADDITIONAL REQUIREMENTS

### Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017).
- **LABELING:** If the labeling of Corticotropin for Injection recommends intravenous administration, include specific information on dosage.
- **USP REFERENCE STANDARDS** (11)  
 USP Ascorbic Acid RS  
 USP Corticotropin RS  
 USP Endotoxin RS  
 USP Vasopressin RS

## Repository Corticotropin Injection

### DEFINITION

Repository Corticotropin Injection is Corticotropin in a solution of partially hydrolyzed gelatin. Its potency is NLT 80.0% and NMT 125.0% of the potency stated on the label in USP Corticotropin Units. It may contain a suitable antimicrobial agent.



**IDENTIFICATION**

- **A.** Meets the requirements of the Assay.

**ASSAY****Change to read:**• **PROCEDURE**

**Standard solution:** Pipet 2.5 mL of gelatin TS into an opened container of USP Corticotropin RS, and mix to obtain a solution having a concentration of 2.0 USP Corticotropin Units/mL. Using gelatin TS as a diluent, prepare three diluted *Standard solutions* such that the respective concentrations of corticotropin constitute a geometric series such as 1:2:4 or 1:3:9 and such that the quantity of corticotropin in each 0.5 mL lies within the range of 10–300 milliunits.

**Sample solution:** In the same manner, using the same diluent, dilute the Injection to give three *Sample solutions* corresponding in concentration to those of the *Standard solutions*.

**The animals:** Select healthy rats, of the same but either sex, that have been raised on a diet fully adequate with respect to vitamin and mineral content. Anesthetize the rats (IRA 1-Jul-2016) and remove the hypophysis from each by application of gentle suction through a fine-tipped tube. Between 16 and 48 h after the operation, select those rats weighing 80–180 g, but restrict the selection so that no rat is more than 30% heavier than the lightest, and the number of rats is an exact multiple of 6. Separate the selected rats into 6 groups, equal in size, of NLT 6 rats each, and assign at random one of the three diluted *Standard solutions* or one of the three *Sample solutions* to each group.

**Analysis:** Inject all rats of each group subcutaneously with the assigned test doses. Three h after the injection, anesthetize the rats, and remove both adrenal glands from each rat, free them from adhering tissue, and promptly weigh each pair on a suitable balance to the nearest 0.2 mg. Place the weighed glands from each rat in suitable vessels each containing 8.0 mL of metaphosphoric acid solution (1 in 40), and pulverize the glands by grinding with a small quantity of washed sand. Cover each vessel, and proceed similarly until all glands have been extracted.

**Ascorbic acid determination:** Filter the metaphosphoric acid extracts, and pipet 4 mL of each filtrate into suitable vessels each containing 4.0 mL of indophenol-acetate TS. Mix by shaking, and read the absorbance at 520 nm, with a suitable spectrophotometer. From the observed absorbance and the standard curve prepared as directed below, calculate the amount of ascorbic acid in mg/100 g of adrenal gland tissue. Prepare a standard concentration-absorbance curve, using three ascorbic acid solutions containing, respectively, 6.0, 8.0, and 10.0 µg/mL of USP Ascorbic Acid RS in metaphosphoric acid solution (1 in 40). Pipet into each of three suitable vessels, preferably spectrophotometer cells, 4 mL of indophenol-acetate TS. Add 4.0 mL of one of the three standard ascorbic acid solutions to one of the cells, mix, and promptly read the absorbance from the same instrument and under the same conditions as for the adrenal gland extracts. Repeat the process for the other two standard ascorbic acid solutions, plot the concentration-absorbance values, and draw the straight line best fitting the three plotted points.

**Calculation:** If there are no missing data; i.e., all groups of rats are the same size,  $f$ , then the following may be used. [NOTE—If there are missing values, then suitable software can be used and standard procedures followed for parallel line bioassays, including assessment of parallelism and linearity.] Tabulate the observed concentration of ascorbic acid in the adrenal glands of each rat, designated by the symbol  $y_{jkl}$ , where  $j = S$  (Standard) or

$U$  (Injection),  $k = 1, 2$ , or 3 for the three doses, and  $l = 1, \dots, f$  rats. Total the values of the  $y_{jkl}$ 's in each group as:

$$T_{jk} = \sum_{l=1}^f y_{jkl}$$

Then determine the following quantities:

$$T_o = \sum_{k=1}^3 (T_{Uk} - T_{Sk})$$

$$T_b = (T_{U3} - T_{U1}) + (T_{S3} - T_{S1})$$

$$V = (T_{U3} - T_{U1}) / (T_{S3} - T_{S1})$$

$$T_q = (T_{U3} - 2T_{U2} + T_{U1}) + (T_{S3} - 2T_{S2} + T_{S1})$$

$$T_{oq} = (T_{U3} - 2T_{U2} + T_{U1}) - (T_{S3} - 2T_{S2} + T_{S1})$$

$$s^2 = \frac{1}{n} \left[ \sum_{j,k,l} y_{jkl}^2 - \frac{1}{f} \sum_{j,k} T_{jk}^2 \right] \text{ where } n = 6(f-1)$$

$$\text{or } = \frac{1}{6} s_b^2 \text{ where } s_b^2 = \frac{1}{f-1} \sum_{l=1}^f (y_{jkl} - \frac{T_{jk}}{f})^2$$

$$F = \frac{T_o^2 + T_{oq}^2}{24fs^2}$$

If  $V \geq 0.75$  and  $V \leq 1.33$ , then the data satisfy parallelism. If  $F \leq F_{0.05,2,n}$ , where  $F_{0.05,2,n}$  is the upper 0.05 percentage point of an  $F$  distribution with 2 and  $n$  degrees of freedom, then the data satisfy linearity. If both conditions are satisfied, determine the logarithm of potency of the Injection,  $M$ , taken as:

$$M = M' + \log R,$$

$$\text{where } M' = 4iT_o/(3T_b)$$

- $i$  = interval between successive log doses of both the *Standard solution* and the *Sample solution*  
 $R$  =  $v_S/v_U$ , the ratio of the high dose of the *Standard solution* in USP Corticotropin Units ( $v_S$ ) to the high dose of the Injection in mL ( $v_U$ )

Determine the width,  $L$ , of the log confidence interval as:

$$L = 2\sqrt{(C-1)(C(M')^2 + \frac{1}{2}i^2)}$$

$$\text{where } C = \frac{T_b^2}{(T_b^2 - 4fs^2t_{0.025,n}^2)}$$

and  $t_{0.025,n}$  is the upper one-sided 0.025 percentage point (or two-sided 0.05 percentage point) of a  $t$ -distribution with  $n$  degrees of freedom.

**Replication:** Repeat the entire determination at least once. Test the agreement among the two or more independent determinations, and compute the weight for each (see *Design and Analysis of Biological Assays* (111), *Combination of Independent Assays*). Calculate the weighted mean log-potency  $\bar{M}$  and its confidence interval,  $L_c$  (see (111), *The Confidence Interval and Limits of Potency*). The potency,  $P$ , is satisfactory if  $P$  = antilog  $\bar{M}$  is 80%–125% of the labeled potency and if the confidence interval does not exceed 0.40.

**Acceptance criteria:** 80.0%–125.0%

**IMPURITIES**• **VASOPRESSIN ACTIVITY**

**Solution A:** Dissolve 6.6 g of dibasic ammonium phosphate in about 950 mL of water, and adjust with con-



centrated phosphoric acid to a pH of 3.0. Dilute with water to 1 L.

**Mobile phase:** Acetonitrile and *Solution A* (13:87). Filter and degas.

[NOTE—The retention time of the vasopressin peak is very sensitive to small changes in the acetonitrile concentration.]

**Standard solution:** Dissolve the entire contents of a vial of USP Vasopressin RS in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 0.1 USP Vasopressin Units/mL.

**Sample solution:** Dissolve the entire contents of a vial of Injection in a known volume of *Solution A*, and dilute with *Solution A* to obtain a final solution containing 2.0 USP Corticotropin Units/mL.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** About 1.5 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the vasopressin activity in USP Vasopressin Units/USP Corticotropin Unit:

$$\text{Result} = C_s \times [(r_u/r_s)/2]$$

$C_s$  = concentration of the *Standard solution* (USP Vasopressin Units/mL)

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

**Acceptance criteria:** NMT 0.05 USP Vasopressin Units/USP Corticotropin Unit

#### SPECIFIC TESTS

• **pH (791):** 3.0–7.0

• **BACTERIAL ENDOTOXINS TEST (85):** NMT 3.1 USP Endotoxin Units/USP Corticotropin Unit

• **INJECTIONS AND IMPLANTED DRUG PRODUCTS (1):** Meets the requirements

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

• **USP REFERENCE STANDARDS (11)**

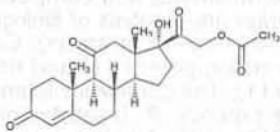
USP Ascorbic Acid RS

USP Corticotropin RS

USP Endotoxin RS

USP Vasopressin RS

## Cortisone Acetate



$C_{23}H_{30}O_6$

402.48

Pregn-4-ene-3,11,20-trione, 21-(acetyloxy)-17-hydroxy-; 17,21-Dihydroxypregn-4-ene-3,11,20-trione 21-acetate [50-04-4].

#### DEFINITION

Cortisone Acetate contains NLT 97.0% and NMT 102.0% of cortisone acetate ( $C_{23}H_{30}O_6$ ), calculated on the dried basis.

#### IDENTIFICATION

##### • A. INFRARED ABSORPTION (197K)

**Sample:** Dissolve in methanol, evaporate the methanol on a steam bath, and dry at 105° for 30 min.

**Acceptance criteria:** Meets the requirements

##### • B. ULTRAVIOLET ABSORPTION (197U)

**Analytical wavelength:** 238 nm

**Medium:** Methanol

**Sample solution:** 10 μg/mL

**Acceptance criteria:** Absorptivities, calculated on the dried basis, do not differ by more than 3.0%.

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Acetonitrile and water (45:55)

**Buffer:** Transfer 20 mL of 1 N hydrochloric acid, 150 mL of 0.5 N potassium chloride, and 50 mL of 0.5 M sodium acetate to a 1-L volumetric flask, and dilute with water to volume.

**Diluent:** Acetonitrile and *Buffer* (1:1)

**Standard solution:** 0.1 mg/mL of USP Cortisone Acetate RS in *Diluent*, prepared as follows. Transfer 25 mg of USP Cortisone Acetate RS to a 250-mL volumetric flask, and dissolve in 100 mL of *Diluent*. Sonicate until a clear solution is obtained, and dilute with *Diluent* to volume.

**Sample solution:** 0.1 mg/mL of Cortisone Acetate in *Diluent*, prepared as follows. Transfer 25 mg of Cortisone Acetate to a 250-mL volumetric flask, and dissolve in 100 mL of *Diluent*. Sonicate until a clear solution is obtained, and dilute with *Diluent* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; 10-μm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Column efficiency:** NLT 1500 theoretical plates

**Tailing factor:** NMT 2.0

**Capacity factor,  $k'$ :** NLT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cortisone acetate ( $C_{23}H_{30}O_6$ ) in the portion of Cortisone Acetate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Cortisone Acetate RS in the *Standard solution* (mg/mL)

$C_u$  = concentration of Cortisone Acetate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 97.0%–102.0% on the dried basis

#### IMPURITIES

• **RESIDUE ON IGNITION (281):** Negligible, from 100 mg

##### • ORGANIC IMPURITIES

**Solution A:** Acetonitrile and water (3:7)

**Solution B:** Acetonitrile and water (7:3)

**Mobile phase:** See *Table 1*.



Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	90	10
25	10	90
30	10	90
31	90	10
51	90	10

**Diluent:** A filtered mixture of acetonitrile, glacial acetic acid, and water (7: 0.1: 3)

**Standard solution:** 20 µg/mL of USP Cortisone Acetate RS in *Diluent*

**Sample solution:** 2.5 mg/mL of Cortisone Acetate in *Diluent*. Sonicate to dissolve.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 15 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Relative standard deviation:** NMT 5.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cortisone Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of the major peak from the *Standard solution*

$C_S$  = concentration of USP Cortisone Acetate RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cortisone Acetate in the *Sample solution* (mg/mL)

#### Acceptance criteria

**Any individual impurity:** NMT 1.5%

**Total impurities:** NMT 2.0%

#### SPECIFIC TESTS

##### • OPTICAL ROTATION, *Specific Rotation* (781S)

**Sample solution:** 10 mg/mL in dioxane

**Acceptance criteria:** +208° to +217°

##### • LOSS ON DRYING (731)

**Sample:** Dry a sample at 105° for 30 min.

**Acceptance criteria:** NMT 1.0%

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE:

Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

##### • USP REFERENCE STANDARDS (11)

USP Cortisone Acetate RS

## Cortisone Acetate Injectable Suspension

» Cortisone Acetate Injectable Suspension is a sterile suspension of Cortisone Acetate in a suitable aqueous medium. It contains not less than 90.0 percent and not more than 110.0 percent of

the labeled amount of cortisone acetate ( $C_{23}H_{30}O_6$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

#### USP Reference standards (11)—

USP Cortisone Acetate RS

**Identification**—Mix 25 mL of water with a volume of Injectable Suspension equivalent to about 25 mg of cortisone acetate. Centrifuge, or allow the insoluble material to settle, then decant and discard the supernatant. Add 20 mL of methanol and, using agitation and warming as necessary, dissolve the residue. Evaporate the solvent on a steam bath with the aid of a current of air, then dry the residue at 105° for 30 minutes; the residue so obtained responds to *Identification test A* under *Cortisone Acetate*.

**pH** (791): between 5.0 and 7.0.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

#### Assay—

**Mobile phase**—Prepare as directed in the *Assay* under *Cortisone Acetate Tablets*.

**Internal standard solution**—Prepare a solution of prednisone in *Mobile phase* having a concentration of 0.5 mg per mL.

**Standard preparation**—Transfer about 12 mg of USP Cortisone Acetate RS, accurately weighed, to a stoppered, 50-mL conical flask. Add 20.0 mL of *Internal standard solution*, and sonicate for 5 minutes. Pass a portion through a polytetrafluoroethylene syringe filter, then combine 1 mL of the filtrate and 4 mL of *Mobile phase* to obtain the *Standard preparation*.

**Resolution solution**—Dissolve a quantity of hydrocortisone acetate in the *Standard preparation* to obtain a solution containing about 0.1 mg of hydrocortisone acetate per mL.

**Assay preparation**—Using a pipet calibrated "to contain," transfer 2.0 mL of freshly mixed Injectable Suspension to a volumetric flask of a size to give a cortisone acetate concentration of 2 mg per mL when diluted to volume. Rinse the suspension remaining in the pipet into the flask with isopropyl alcohol, dilute with isopropyl alcohol to volume, and sonicate for 3 minutes. Deliver a 3.0-mL aliquot of this solution to a stoppered, 25-mL conical flask, and evaporate on a steam bath with the aid of a current of air to dryness. Add 10.0 mL of *Internal standard solution*, insert the stopper, and sonicate for 5 minutes. Pass a portion through a polytetrafluoroethylene syringe filter, then combine approximately 1 mL of the filtrate and 4 mL of *Mobile phase* to obtain the *Assay preparation*.

**Chromatographic system** (see *Chromatography* (621))—Prepare as directed in the *Assay* under *Cortisone Acetate Tablets*. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the resolution,  $R$ , between cortisone acetate and hydrocortisone acetate is not less than 2.2 (if necessary, add equal parts of *n*-butyl chloride and water-saturated *n*-butyl chloride to the *Mobile phase* to meet this requirement). Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are 0.6 for cortisone acetate and 1.0 for prednisone; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Proceed as directed in the *Assay* under *Cortisone Acetate Tablets*. Calculate the quantity, in mg, of cortisone acetate ( $C_{23}H_{30}O_6$ ) in each mL of the Injectable Suspension taken by the formula:

$$W(V/12)(R_U/R_S)$$

in which  $V$  is the capacity, in mL, of the volumetric flask used for the *Assay preparation*; and the other terms are as defined therein.



## Cortisone Acetate Tablets

### DEFINITION

Cortisone Acetate Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cortisone acetate ( $C_{23}H_{30}O_6$ ).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (17K)

**Sample:** Powder a number of Tablets equivalent to 25 mg of cortisone acetate. Add 25 mL of solvent hexane, and extract for 15 min with occasional agitation. Decant and discard the supernatant, then extract the residue with 5 mL of chloroform, with frequent agitation, for 5 min. Filter, add 10 mL of methanol to the filtrate, mix, evaporate the solvent on a steam bath with the aid of a current of air, then dry the residue at 105° for 30 min. Use the residue.

**Acceptance criteria:** Meet the requirements

### ASSAY

#### • PROCEDURE

**Mobile phase:** *n*-Butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol, and glacial acetic acid (95:95:14:7:6)

**Internal standard solution:** 0.04 mg/mL of methylparaben in *Mobile phase*

**Standard stock solution:** Transfer 12 mg of USP Cortisone Acetate RS to a glass-stoppered, 50-mL conical flask, add 25.0 mL of the *Internal standard solution*, and sonicate for 5 min.

**Standard solution:** Nominally 0.12 mg/mL of USP Cortisone Acetate RS, prepared by combining 1 mL of the *Standard stock solution* with 3 mL of *Mobile phase*

**System suitability solution:** 0.1 mg/mL of hydrocortisone acetate in the *Standard solution*

**Sample stock solution:** Weigh, then finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 12 mg of cortisone acetate, to a stoppered conical flask. Add 25.0 mL of the *Internal standard solution*, insert the stopper into the flask, and sonicate vigorously for 5 min. Pass a portion through a polytetrafluoroethylene syringe filter.

**Sample solution:** 0.12 mg/mL of cortisone acetate, prepared by combining 1 mL of the *Sample stock solution* filtrate and 3 mL of *Mobile phase*

#### Chromatographic system

(See Chromatography (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L3

**Flow rate:** 1 mL/min

**Injection volume:** 15 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for methylparaben and cortisone acetate are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.2 between cortisone acetate and hydrocortisone acetate, *System suitability solution*. If necessary, add equal parts of *n*-butyl chloride and water-saturated *n*-butyl chloride to the *Mobile phase* to meet this requirement.

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cortisone acetate ( $C_{23}H_{30}O_6$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of cortisone acetate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of cortisone acetate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Cortisone Acetate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cortisone acetate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Medium:** 0.5% sodium lauryl sulfate solution; 1000 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Standard solution:** 5.55 µg/mL of USP Cortisone Acetate RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. Dilute with *Medium*, if necessary.

#### Instrumental conditions

**Mode:** UV

**Analytical wavelength:** Maximum absorbance at about 242 nm

**Cell size:** 1 cm

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

**Tolerances:** NLT 75% (Q) of the labeled amount of cortisone acetate ( $C_{23}H_{30}O_6$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905)

##### Procedure for content uniformity

**Mobile phase, Internal standard solution, Standard solution, System suitability solution, Chromatographic system, and System suitability:** Proceed as directed in the *Assay*.

**Sample stock solution:** Place 1 Tablet in a stoppered, 50-mL conical flask, deposit 0.25 mL of water onto the Tablet, insert the stopper into the flask, and allow to stand for 30 min. Add 2.5 mL of isopropyl alcohol, and place the unstoppered flask on a steam bath. Boil gently, if necessary, until the Tablet disintegrates, then evaporate the solvent with the aid of a current of air. Remove from the steam bath, add 10.0 mL of the *Internal standard solution* for each 5 mg of cortisone acetate declared, insert the stopper, and sonicate vigorously for 10 min. Pass a portion through a polytetrafluoroethylene syringe filter.

**Sample solution:** Combine 1 mL of the *Sample stock solution* filtrate and 3 mL of *Mobile phase*.

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cortisone acetate ( $C_{23}H_{30}O_6$ ) in the Tablet taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of cortisone acetate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of cortisone acetate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Cortisone Acetate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cortisone acetate in the *Sample solution* (mg/mL)

**Acceptance criteria:** Meet the requirements

### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in well-closed containers.

• **USP REFERENCE STANDARDS (11)**

USP Cortisone Acetate RS



## Cosyntropin

SYNHEFRWG KPVGKRRPV KVYP

$C_{136}H_{210}N_{40}O_{31}S$   
 $\alpha^1$ -<sup>24</sup>-Corticotropin [16960-16-0].

2933

### DEFINITION

Cosyntropin is a synthetic peptide whose sequence is identical to the first 24 amino acids of human adrenocorticotrophic hormone (ACTH). Cosyntropin contains NLT 90% and NMT 102% of cosyntropin ( $C_{136}H_{210}N_{40}O_{31}S$ ), calculated on the anhydrous, acetic acid-free basis.

### IDENTIFICATION

- **A.** The retention time of the cosyntropin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. AMINO ACID ANALYSIS**  
**Hydrolysis solution:** 12 N hydrochloric acid containing a small crystal (0.1%–1.0%) of phenol  
**Sample hydrolysate preparation:** Transfer a portion of cosyntropin into a vial, weigh it, and dissolve in water to a concentration of 3 mg/mL. Transfer 70 mg of this solution to a vacuum hydrolysis tube. Add 70  $\mu$ L of *Hydrolysis solution*, seal the tube, and heat for 24 h at 110°. Cool the tube, and remove the solvents at reduced pressure. Resuspend the hydrolysate residue in 1 mL of 0.020 M hydrochloric acid, and filter.  
**Solution A:** Prepare a solution having a composition of 140 mM sodium acetate and 17 mM triethylamine, and adjust with phosphoric acid to a pH of 5.02.<sup>1</sup>  
**Solution B:** Acetonitrile and water (60:40)  
**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
0.5	99	1
18	95	5
19	91	9
29.5	77	23
40	77	23
40.01	40	60
50	40	60

**Sample solution:** Add 140  $\mu$ L of 0.2 M borate buffer (pH 8.8) to 20  $\mu$ L of *Sample hydrolysate preparation*. Add 40  $\mu$ L of 10 mM 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). Incubate for 2 min at room temperature. Transfer to an insert in an HPLC vial, seal with a silicone cap, and incubate for 10 min at 55°.

**Standard solution:** Prepare a solution having known equimolar amounts of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine with half the equimolar amount of L-cystine. [NOTE—Suitable concentrations are 0.1 mM and 0.05 mM, respectively.] Derivatize with AQC as outlined for *Sample solution*.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

<sup>1</sup> A suitable substitute is Eluant A from Waters Corporation, catalog number WAT052890.

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  15-cm; 4- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Samples:** *Sample solution* and *Standard solution*

**Suitability requirements:** All 17 amino acid peaks must be visible in the *Standard solution*.

**Resolution:** NLT 1.5 between each of the 17 amino acid peaks in the *Standard solution*. [NOTE—In those cases where the peak does not recover to the baseline, the following criteria will be applied:  $(hp - hv)/hp \times 100 \pm 90\%$ , where  $hp$  is the height of the minor peak, and  $hv$  is the height of the valley between the peaks.]

#### Analysis

**Samples:** *Sample solution* and *Standard solution*

First record and measure the responses for each amino acid peak in the *Standard solution*. Express the content of each amino acid in moles.

Calculate the mean nmol of the amino acids:

$$\text{Result} = (\text{nmol found in the Analysis for Glu, Gly, Val, Phe, Lys, Arg, Pro})/17$$

Divide the nmol of each amino acid by the *Result* to determine the amino acid ratios that must meet the *Acceptance criteria*.

**Acceptance criteria:** 3.5–4.7 of lysine; 0.9–1.1 of histidine; 2.7–3.3 of arginine; 1.1–2.2 of serine; 0.9–1.1 of glutamic acid; 2.5–3.5 of proline; 1.8–2.2 of glycine; 0.9–1.1 of methionine; 1.7–2.2 of tyrosine; 0.9–1.1 of phenylalanine; 2.7–3.3 of valine

### ASSAY

#### PROCEDURE

**Mobile phase:** 365 mL of acetonitrile, 10 mL of glacial acetic acid, and 10 g of ammonium sulfate. Dilute with water to 2 L, and a pH of about 3.3.

**Standard solution:** 1.0 mg/mL of USP Cosyntropin Acetate RS

**Sample solution:** 1.0 mg/mL of Cosyntropin

**Chromatographic system**  
 (See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm  $\times$  25-cm; 5- $\mu$ m packing L1

**Column temperature:** 40°

**Flow rate:** 1.0 mL/min

**Injection volume:** 50  $\mu$ L

**Run time:** 50 min

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.0 between the main cosyntropin peak and the reduced Trp-cosyntropin peak in the *Standard solution*

**Retention time:** 16–20 min for the cosyntropin peak, *Standard solution*

**Relative standard deviation:** NMT 2.0% for the cosyntropin peak from three replicate injections of the *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cosyntropin ( $C_{136}H_{210}N_{40}O_{31}S$ ) in the portion of Cosyntropin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cosyntropin from the *Sample solution*

$r_S$  = peak response of cosyntropin from the *Standard solution*



- $C_S$  = concentration of cosyntropin in each vial of USP Cosyntropin Acetate RS in the *Standard solution* (mg/mL)
- $C_U$  = concentration of Cosyntropin, calculated on the anhydrous, acetic acid-free basis, in the *Sample solution* (mg/mL)
- Acceptance criteria: 90.0%–102.0% of cosyntropin on the anhydrous, acetic acid-free basis

**IMPURITIES**• **ORGANIC IMPURITIES, RELATED PEPTIDES**

Mobile phase, *Standard solution*, *Sample solution*, and *Chromatographic system*: Proceed as directed in the *Assay*.

Peak identification solution: 1.0 mg/mL of cosyntropin in 1% (v/v) glacial acetic acid. Add 50  $\mu$ L of 30% hydrogen peroxide:water (1:999). Let stand for 2 h to produce the main impurity, cosyntropin sulfoxide.

**System suitability**

*Samples*: *Standard solution* and *Peak identification solution*

**Suitability requirements**

**Resolution**: NLT 1.0 between the main cosyntropin peak and the reduced Trp-cosyntropin peak in the *Standard solution*

**Retention time**: 16–20 min for the cosyntropin peak, *Standard solution*

**Relative retention time of main impurity in *Peak identification solution***: About 0.4

**Relative standard deviation**: NMT 2.0% for the cosyntropin peak from three replicate injections of the *Standard solution*

**Analysis**

*Sample*: *Sample solution*

Integrate the chromatogram using the normalization procedure.

Calculate the percentage of cosyntropin-related impurities in the portion of Cosyntropin taken:

$$\text{Result} = (r_i/r_T) \times 100$$

$r_i$  = peak response of any individual impurity from the *Sample solution*

$r_T$  = sum of all peak responses from the *Sample solution*

**Acceptance criteria**

**Individual impurities**: See Table 2.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cosyntropin sulfoxide	0.4	2
Reduced Trp in cosyntropin	0.9	2
Any other individual impurity	—	1
Total impurities	—	5

**OTHER COMPONENTS**

- **ACETIC ACID IN PEPTIDES** (503): 8%–13% using a 1-mg/mL *Test Solution* prepared by dissolving 10 mg of Cosyntropin in 10 mL of *Solution A* and *Solution B* (95:5)
- **TRIFLUOROACETIC ACID CONTENT**: Perform the method contained in (503), substituting a suitable quantity of trifluoroacetic acid for the *Standard solution*. The portion of Cosyntropin taken shall contain NMT 0.5% trifluoroacetic acid.

**SPECIFIC TESTS**• **UV ABSORPTION SPECTROPHOTOMETRY**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Wavelength range**: 240–280 nm

**Sample solution**: 0.2 mg/mL in 0.1 M hydrochloric acid

**Acceptance criteria**: Absorptivity of 0.51–0.61, calculated on the anhydrous and acetic acid-free basis, at the maximum wavelength of 276 nm

**Ratio**: A276/A248, 2.4–2.9

- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 10 USP Endotoxin Units/mg of cosyntropin.
- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIED MICROORGANISMS** (62): The total aerobic microbial count is less than 100 cfu/g.
- **WATER DETERMINATION, Method 1c** (921): NMT 10.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers at 2°–8°.
- **USP REFERENCE STANDARDS** (11)  
USP Cosyntropin Acetate RS  
USP Endotoxin RS

**Purified Cotton**

» Purified Cotton is the hair of the seed of cultivated varieties of *Gossypium hirsutum* Linné, or of other species of *Gossypium* (Fam. Malvaceae), freed from adhering impurities, deprived of fatty matter, bleached, and sterilized in its final container.

**Packaging and storage**—Package it in rolls of not more than 500 g of a continuous lap, with a light-weight paper running under the entire lap, the paper being of such width that it may be folded over the edges of the lap to a distance of at least 25 mm, the two together being tightly and evenly rolled, and enclosed and sealed in a well-closed container. It may be packaged also in other types of containers if these are so constructed that the sterility of the product is maintained.

**Labeling**—Its label bears a statement to the effect that the sterility cannot be guaranteed if the package bears evidence of damage or if the package has been opened previously.

**Sterility Tests** (71): meets the requirements.

**Acidity or alkalinity**—Thoroughly saturate about 10 g with 100 mL of recently boiled and cooled water, then with the aid of a glass rod press out two 25-mL portions of the water into white porcelain dishes. To one portion add 3 drops of phenolphthalein TS, and to the other portion add 1 drop of methyl orange TS: no pink color develops in either portion.

**Residue on ignition** (281)—Place about 5 g, accurately weighed, in a porcelain or platinum dish, and moisten with 2 N sulfuric acid. Gently heat the cotton until it is charred, then ignite more strongly until the carbon is completely consumed: not more than 0.20% of residue remains.

**Water-soluble substances**—Place 10 g, accurately weighed, in a beaker containing 1000 mL of water, and boil gently for 30 minutes, adding water as required to maintain the volume. Pour the water through a funnel into another vessel, and press out the excess water from the cotton with a glass rod. Wash the cotton in the funnel with two 250-mL portions of boiling water, pressing the cotton after each washing. Filter the combined extract and washings, and wash the filter thoroughly with hot water. Evaporate the combined extract and washings to a small volume, transfer to a tared porcelain or platinum dish, evaporate to dryness, and dry the residue at 105° to constant weight: the residue weighs not more than 35 mg (0.35%).



**Fatty matter**—Pack  $10 \pm 0.01$  g in a Soxhlet extractor provided with a tared receiver, and extract with ether for 5 hours at a rate such that the ether siphons over not less than four times per hour. The ether solution in the flask shows no trace of blue, green, or brownish color. Evaporate the extract to dryness, and dry at  $105^\circ$  for 1 hour: the weight of the residue does not exceed 70 mg (0.7%).

**Dyes**—Pack about 10 g in a narrow percolator, and extract slowly with alcohol until the percolate measures 50 mL: when observed downward through a column 20 cm in depth, the percolate may show a yellowish color, but neither a blue nor a green tint.

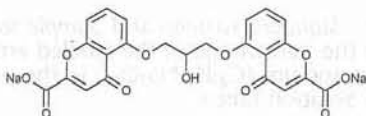
**Other foreign matter**—The pinches of it taken for the determination of *Fiber length* contain no oil stains or metallic particles.

**Fiber length and Absorbency**—Remove it from its wrappings, and condition it for not less than 4 hours in a standard atmosphere of  $65 \pm 2\%$  relative humidity at  $21 \pm 1.1^\circ$  ( $70 \pm 2^\circ\text{F}$ ), before determining the *Fiber length* and *Absorbency*.

**Fiber length**—Determine the fiber length of Purified Cotton as directed under *Cotton—Fiber Length* (691): not less than 60% of the fibers, by weight, are 12.5 mm or greater in length, and not more than 10% of the fibers, by weight, are 6.25 mm or less in length.

**Absorbency**—Proceed as directed under *Cotton—Absorbency Test* (691): submersion is complete in 10 seconds at a temperature of  $25^\circ$ , and the cotton retains not less than 24 times its weight of water.

## Cromolyn Sodium



$\text{C}_{23}\text{H}_{14}\text{Na}_2\text{O}_{11}$  512.33

4*H*-1-Benzopyran-2-carboxylic acid, 5,5'-[(2-hydroxy-1,3-propanediyl)bis(oxy)bis[4-oxo-, disodium salt].

Disodium 5,5'-[(2-hydroxytrimethylene)dioxy]bis[4-oxo-4*H*-1-benzopyran-2-carboxylate] [15826-37-6].

» Cromolyn Sodium contains not less than 98.0 percent and not more than 101.0 percent of  $\text{C}_{23}\text{H}_{14}\text{Na}_2\text{O}_{11}$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cromolyn Sodium RS

**Identification**—

**A:** The IR absorption spectrum of a potassium bromide dispersion of it, previously dried in vacuum at  $105^\circ$  to constant weight, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Cromolyn Sodium RS.

**B:** The UV absorption spectrum of a 1 in 40,000 solution in pH 7.4 sodium phosphate buffer prepared as directed in the Assay exhibits maxima at the same wavelengths as that of a similar solution of USP Cromolyn Sodium RS, concomitantly measured.

**Acidity or alkalinity**—Dissolve 1.0 g in 25 mL of carbon dioxide-free water, and add two drops of bromothymol blue TS. If the solution is yellow, not more than 0.25 mL of 0.1 N sodium hydroxide is required to produce a blue color. If the solution is blue, not more than 0.25 mL of 0.1 N hydrochloric acid is required to produce a yellow color.

**Water Determination, Method I** (921): not more than 10.0%.

**Delete the following:**

• **Heavy metals, Method II** (231): 0.002%. • (Official 1-Jan-2018)

**Limit of oxalate**—Dissolve 100 mg in 20 mL of water, add 5.0 mL of iron salicylate TS, and dilute with water to 50 mL. Determine the absorbance of the solution at 480 nm against a water blank. The absorbance is not less than that of a solution containing  $350 \mu\text{g}$  of oxalic acid prepared in the same manner (0.35%).

**Related compounds**—Dissolve 100 mg of Cromolyn Sodium in 10.0 mL of a mixture of water, stabilizer-free tetrahydrofuran, and acetone (6:4:1). Similarly prepare a solution of USP Cromolyn Sodium RS in the same solvent mixture having a concentration of 10 mg per mL (*Standard solution A*). Quantitatively dilute a volume of *Standard solution A* with the same solvent mixture to obtain a diluted standard solution having a concentration of 0.05 mg per mL (*Standard solution B*). Apply 10- $\mu\text{L}$  portions of the test solution, *Standard solution A*, and *Standard solution B* to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, methanol, and glacial acetic acid (9:9:2) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from *Standard solution A*. Any spot in the chromatogram obtained from the test solution moving ahead of the principal spot is not more intense than the spot in the chromatogram obtained from *Standard solution B* (0.5%).

**Assay**—

**pH 7.4 Sodium phosphate buffer**—Dissolve 70 g of anhydrous dibasic sodium phosphate in 900 mL of water. Adjust to a pH of 7.4 by the addition of dilute phosphoric acid (1 in 10). Dilute with water to 1000 mL, and mix. Transfer 10 mL of this solution to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cromolyn Sodium RS in water to obtain a solution having a known concentration of about  $250 \mu\text{g}$  per mL. Transfer 10 mL of this solution to a 100-mL volumetric flask, add 1 mL of pH 7.4 Sodium phosphate buffer, dilute with water to volume, and mix. The final concentration is about  $25 \mu\text{g}$  per mL.

**Assay preparation**—Transfer about 100 mg of Cromolyn Sodium, accurately weighed, to a 1000-mL volumetric flask, dissolve in about 100 mL of water, dilute with water to volume, and mix. Pipet 25 mL of this solution into a 100-mL volumetric flask, add 1 mL of pH 7.4 Sodium phosphate buffer, dilute with water to volume, and mix.

**Procedure**—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 326 nm, with a suitable spectrophotometer, using a 1 in 100 solution of pH 7.4 Sodium phosphate buffer as the blank. Calculate the quantity, in mg, of  $\text{C}_{23}\text{H}_{14}\text{Na}_2\text{O}_{11}$  in the Cromolyn Sodium taken by the formula:

$$4C(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Cromolyn Sodium RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the solutions from the *Assay preparation* and the *Standard preparation*, respectively.



## Cromolyn Sodium Inhalation Powder

» Cromolyn Sodium Inhalation Powder is a mixture of equal parts of Lactose and Cromolyn Sodium contained in a hard gelatin capsule. It contains not less than 95.0 percent and not more than 125.0 percent of the labeled amount of cromolyn sodium ( $C_{23}H_{14}Na_2O_{11}$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers, and store at controlled room temperature. Avoid excessive heat.

**USP Reference standards** (11)—  
USP Cromolyn Sodium RS

**Identification**—It meets the requirements of *Identification test B* under *Cromolyn Sodium*.

**Uniformity of dosage units** (905): meets the requirements.

### Assay—

*pH 7.4 Sodium phosphate buffer*—Prepare as directed in the *Assay* under *Cromolyn Sodium*.

*Assay preparation*—Remove and accurately weigh the contents of not fewer than 20 capsules of Cromolyn Sodium Inhalation Powder, and transfer the combined contents to a 250-mL volumetric flask. Dissolve in 100 mL of water, dilute with water to volume, and mix. Transfer an aliquot of this solution, equivalent to 8 mg of cromolyn sodium, to a 250-mL volumetric flask, add 1 mL of *pH 7.4 Sodium phosphate buffer*, dilute with water to volume, and mix.

*Standard preparation*—Dissolve a suitable quantity of USP Cromolyn Sodium RS, previously dried in vacuum at 105° to constant weight and accurately weighed, in water to obtain a solution having a known concentration of about 350 µg per mL. Transfer 10 mL of this solution to a 100-mL volumetric flask, add 1 mL of *pH 7.4 Sodium phosphate buffer*, dilute with water to volume, and mix. The final concentration is about 35 µg per mL.

*Procedure*—Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* in 1-cm cells at the wavelength of maximum absorbance at about 326 nm, with a suitable spectrophotometer, using a solution of *pH 7.4 Sodium phosphate buffer* (1 in 250) as the blank. Calculate the quantity, in mg, of cromolyn sodium ( $C_{23}H_{14}Na_2O_{11}$ ) in the aliquot taken by the formula:

$$0.25C(A_U / A_S)$$

in which *C* is the concentration, in µg per mL, of USP Cromolyn Sodium RS in the *Standard preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Cromolyn Sodium Inhalation Solution

### DEFINITION

Cromolyn Sodium Inhalation Solution is a sterile, aqueous solution of Cromolyn Sodium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cromolyn sodium ( $C_{23}H_{14}Na_2O_{11}$ ).

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** 5.6 g/L of monobasic potassium phosphate and 22.2 g/L of myristyltrimethylammonium bromide in water. Adjust with sodium hydroxide solution to a pH of 6.5.

**Mobile phase:** Methanol and *Buffer* (55:45)

**Diluent:** Acetonitrile and water (30:70)

**System suitability solution:** 0.5 mg/mL of USP

Cromolyn Sodium RS and 0.02 mg/mL each of USP

Cromolyn Related Compound A RS and USP Cromolyn Related Compound B RS in *Diluent*

**Standard solution:** 0.5 mg/mL of USP Cromolyn Sodium RS in *Diluent*

**Sample solution:** Nominally equivalent to 0.5 mg/mL of cromolyn sodium from a volume of Inhalation Solution in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 326 nm

**Column:** 4.6-mm × 10-cm; 3.5-µm packing L7

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between cromolyn related compound B and cromolyn related compound A, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cromolyn sodium ( $C_{23}H_{14}Na_2O_{11}$ ) in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cromolyn from the *Sample solution*

$r_S$  = peak response of cromolyn from the *Standard solution*

$C_S$  = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

### IMPURITIES

#### • ORGANIC IMPURITIES

**Buffer, Mobile phase, Diluent, and System suitability solution:** Proceed as directed in the *Assay*.

**Standard solution:** 0.002 mg/mL each of USP

Cromolyn Related Compound A RS, USP Cromolyn Related Compound B RS, and USP Cromolyn Sodium RS in *Diluent*

**Sample solution:** Nominally equivalent to 2 mg/mL of cromolyn sodium from a volume of Inhalation Solution in *Diluent*



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 326 nm

Column: 4.6-mm × 10-cm; 3.5-μm packing L7

Temperatures

Column: 40°

Autosampler: 4°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

Run time: 2 times the retention time of cromolyn

**System suitability**Samples: *System suitability solution* and *Standard solution*[NOTE—See *Table 1* for the relative retention times.]**Suitability requirements**Resolution: NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn, *System suitability solution*Relative standard deviation: NMT 3% for six replicate injections, *Standard solution***Analysis**Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cromolyn related compound A or cromolyn related compound B in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cromolyn related compound A or cromolyn related compound B from the *Sample solution*

$r_S$  = peak response of cromolyn related compound A or cromolyn related compound B from the *Standard solution*

$C_S$  = concentration of the corresponding cromolyn related compound in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Inhalation Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each unspecified degradation product from the *Sample solution*

$r_S$  = peak response of cromolyn from the *Standard solution*

$C_S$  = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See *Table 1*.**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cromolyn related compound B	0.4	1.0
Cromolyn related compound A	0.5	1.0
Cromolyn	1.0	—
Any unspecified individual degradation product	—	1.0
Total impurities	—	2.0

**SPECIFIC TESTS**• **PH (791):** 4.0–7.0• **STERILITY TESTS (71):** Meets the requirements**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in single-unit double-ended glass ampuls or in low-density polyethylene ampuls. Store at controlled room temperature, protected from light.• **LABELING:** The label indicates that the Inhalation Solution is not to be used if it contains a precipitate.• **USP REFERENCE STANDARDS (11)**

USP Cromolyn Related Compound A RS

1,3-Bis(2-acetyl-3-hydroxyphenoxy) propan-2-ol.

 $C_{19}H_{20}O_7$  360.36

USP Cromolyn Related Compound B RS

Diethyl 5,5'-[(2-hydroxypropane-1,3-diyl)bis(ox-y)]bis(4-oxo-4H-chromene-2-carboxylate).

 $C_{27}H_{24}O_{11}$  524.48

USP Cromolyn Sodium RS

**Cromolyn Sodium Nasal Solution****DEFINITION**Cromolyn Sodium Nasal Solution is an aqueous solution of Cromolyn Sodium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cromolyn sodium ( $C_{23}H_{14}Na_2O_{11}$ ). It may contain suitable stabilizers.**IDENTIFICATION**• **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.**ASSAY**• **PROCEDURE****Buffer:** 5.6 g/L of monobasic potassium phosphate and 22.2 g/L of myristyltrimethylammonium bromide in water. Adjust with sodium hydroxide solution to a pH of 6.5.**Mobile phase:** Methanol and *Buffer* (55:45)**Diluent:** Acetonitrile and water (30:70)**System suitability solution:** 0.5 mg/mL of USP Cromolyn Sodium RS and 0.02 mg/mL each of USP Cromolyn Related Compound A RS and USP Cromolyn Related Compound B RS in *Diluent***Standard solution:** 0.5 mg/mL of USP Cromolyn Sodium RS in *Diluent*. Sonication may be needed to aid dissolution.**Sample solution:** Nominally equivalent to 0.5 mg/mL of cromolyn sodium from a volume of Nasal Solution in *Diluent***Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 326 nm

Column: 4.6-mm × 10-cm; 3.5-μm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 μL

**System suitability**Samples: *System suitability solution* and *Standard solution*[NOTE—See *Table 1* for relative retention times.]**Suitability requirements**Resolution: NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn, *System suitability solution*Tailing factor: NMT 2.0, *Standard solution*Relative standard deviation: NMT 0.73%, *Standard solution*



**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of the labeled amount of cromolyn sodium ( $C_{23}H_{14}Na_2O_{11}$ ) in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of cromolyn from the *Sample solution*  
 $r_S$  = peak response of cromolyn from the *Standard solution*  
 $C_S$  = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**IMPURITIES**• **ORGANIC IMPURITIES**

**Mobile phase, Diluent, and System suitability solution:** Proceed as directed in the Assay.

**Standard solution:** 0.002 mg/mL each of USP Cromolyn Related Compound A RS, USP Cromolyn Related Compound B RS, and USP Cromolyn Sodium RS in *Diluent*

**Sample solution:** Nominally equivalent to 2 mg/mL of cromolyn sodium from a volume of Nasal Solution in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 326 nm

Column: 4.6-mm  $\times$  10-cm; 3.5- $\mu$ m packing L7

Temperatures

Autosampler: 4°

Column: 40°

Flow rate: 1.5 mL/min

Injection volume: 20  $\mu$ L

Run time: 2 times the retention time of cromolyn

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn, *System suitability solution*

**Relative standard deviation:** NMT 3% for six replicate injections for each peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cromolyn related compound A or cromolyn related compound B in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of cromolyn related compound A or cromolyn related compound B from the *Sample solution*  
 $r_S$  = peak response of cromolyn related compound A or cromolyn related compound B from the *Standard solution*  
 $C_S$  = concentration of the corresponding cromolyn related compound in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Nasal Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response of each unspecified degradation product from the *Sample solution*  
 $r_S$  = peak response of cromolyn from the *Standard solution*  
 $C_S$  = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cromolyn related compound B	0.4	1.0
Cromolyn related compound A	0.5	1.0
Cromolyn	1.0	—
Any unspecified individual degradation product	—	1.0
Total impurities	—	2.0

**SPECIFIC TESTS**

- **pH (791):** 4.0–7.0

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
 USP Cromolyn Related Compound A RS  
 1,3-Bis(2-acetyl-3-hydroxyphenoxy) propan-2-ol.  
 $C_{19}H_{20}O_7$  360.36  
 USP Cromolyn Related Compound B RS  
 Diethyl 5,5'-[(2-hydroxypropane-1,3-diyl)bis(ox-  
 y)]bis(4-oxo-4H-chromene-2-carboxylate).  
 $C_{27}H_{24}O_{11}$  524.48  
 USP Cromolyn Sodium RS

**Cromolyn Sodium Ophthalmic Solution****DEFINITION**

Cromolyn Sodium Ophthalmic Solution is a sterile, aqueous solution of Cromolyn Sodium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cromolyn sodium ( $C_{23}H_{14}Na_2O_{11}$ ). It may contain suitable antimicrobial and stabilizing agents.

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Buffer:** 5.6 g/L of monobasic potassium phosphate and 22.2 g/L of myristyltrimethylammonium bromide in water. Adjust with sodium hydroxide solution to a pH of 6.5.

**Mobile phase:** Methanol and *Buffer* (55:45)

**Diluent:** Acetonitrile and water (30:70)

**System suitability solution:** 0.5 mg/mL of USP

Cromolyn Sodium RS and 0.02 mg/mL each of USP Cromolyn Related Compound A RS and USP Cromolyn Related Compound B RS in *Diluent*

**Standard solution:** 0.5 mg/mL of USP Cromolyn Sodium RS in *Diluent*. Sonication may be needed to aid dissolution.



**Sample solution:** Nominally equivalent to 0.5 mg/mL of cromolyn sodium from a volume of Ophthalmic Solution in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 326 nm

**Column:** 4.6-mm × 10-cm; 3.5-μm packing L7

**Column temperature:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—See *Table 1* for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn sodium, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cromolyn sodium (C<sub>23</sub>H<sub>14</sub>Na<sub>2</sub>O<sub>11</sub>) in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cromolyn from the *Sample solution*

$r_S$  = peak response of cromolyn from the *Standard solution*

$C_S$  = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Mobile phase, Diluent, and System suitability solution:** Proceed as directed in the *Assay*.

**Standard solution:** 0.002 mg/mL each of USP Cromolyn Related Compound B RS, USP Cromolyn Related Compound A RS, and USP Cromolyn Sodium RS in *Diluent*

**Sample solution:** Nominally equivalent to 2 mg/mL of cromolyn sodium from a volume of Ophthalmic Solution in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 326 nm

**Column:** 4.6-mm × 10-cm; 3.5-μm packing L7

**Temperatures**

**Autosampler:** 4°

**Column:** 40°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

**Run time:** 2 times the retention time of cromolyn

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between cromolyn related compound B and cromolyn related compound A; NLT 2.0 between cromolyn related compound A and cromolyn sodium, *System suitability solution*

**Relative standard deviation:** NMT 3% for six replicate injections, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each cromolyn related compound in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cromolyn related compound A or cromolyn related compound B from the *Sample solution*

$r_S$  = peak response of cromolyn related compound A or cromolyn related compound B from the *Standard solution*

$C_S$  = concentration of the corresponding cromolyn related compound in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unspecified degradation product in the portion of Ophthalmic Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each unspecified degradation product from the *Sample solution*

$r_S$  = peak response of cromolyn from the *Standard solution*

$C_S$  = concentration of USP Cromolyn Sodium RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cromolyn sodium in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 1*.

**Table 1**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cromolyn related compound B	0.4	0.75
Cromolyn related compound A	0.5	0.75
Cromolyn sodium	1.0	—
Any individual unspecified degradation product	—	0.75
Total impurities	—	2.0

#### SPECIFIC TESTS

• **pH (791):** 4.0–7.0

• **STERILITY TESTS (71):** Meets the requirements

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant, single-dose or multiple-dose containers. Ophthalmic Solution that is packaged in multiple-dose containers contains a suitable antimicrobial agent. Store between 15° and 30°.

#### • USP REFERENCE STANDARDS (11)

USP Cromolyn Related Compound A RS  
1,3-Bis(2-acetyl-3-hydroxyphenoxy) propan-2-ol.

C<sub>19</sub>H<sub>20</sub>O<sub>7</sub> 360.36

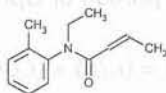
USP Cromolyn Related Compound B RS  
Diethyl 5,5'-[(2-hydroxypropane-1,3-diyl)bis(ox-  
y)]bis(4-oxo-4H-chromene-2-carboxylate).

C<sub>27</sub>H<sub>24</sub>O<sub>11</sub> 524.48

USP Cromolyn Sodium RS



## Crotamiton



$C_{13}H_{17}NO$  203.28  
2-Butenamide, *N*-ethyl-*N*-(2-methylphenyl)-  
*N*-Ethyl-*o*-crotonotoluidide [483-63-6].

» Crotamiton is a mixture of *cis* and *trans* isomers containing not less than 97.0 percent and not more than 103.0 percent of  $C_{13}H_{17}NO$ .

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—  
USP Crotamiton RS

### Identification—

**A: Infrared Absorption** (197F).

**B: Ultraviolet Absorption** (197U)—

**Solution:** 20  $\mu$ g per mL.

**Medium:** cyclohexane.

**C:** To about 10 mL of a saturated solution in water add a few drops of potassium permanganate TS: a brown color is produced, and a brown precipitate is formed on standing.

**Specific gravity** (841): between 1.008 and 1.011 at 20°.

**Refractive index** (831): between 1.540 and 1.543 at 20°.

**Residue on ignition** (281): not more than 0.1%.

**Bound halogen**—Place 4 drops in a 3-mm (ID) test tube, and add calcium oxide to a height of 1 cm. Heat the tube in a flame, starting from the top, until the reaction is complete, then ignite for a short time. Transfer the contents to a beaker containing 10 mL of water, acidify with nitric acid, and filter. To the filtrate add 0.2 mL of silver nitrate solution (1 in 60): any opalescence obtained is not more than that obtained from a blank solution treated in the same manner.

**Assay**—Transfer about 50 mg of Crotamiton, accurately weighed, to a 100-mL volumetric flask, add cyclohexane to volume, and mix. Transfer 10.0 mL of this solution to a 250-mL volumetric flask, dilute with cyclohexane to volume, and mix. Determine the absorbance of this solution and of a solution of USP Crotamiton RS in the same medium having a known concentration of about 20  $\mu$ g per mL in 1-cm cells at the wavelength of maximum absorbance at about 242 nm, with a suitable spectrophotometer, using cyclohexane as the blank. Calculate the quantity, in mg, of  $C_{13}H_{17}NO$  in the Crotamiton taken by the formula:

$$2.5C(A_U / A_S)$$

in which *C* is the concentration, in  $\mu$ g per mL, of USP Crotamiton RS in the Standard solution; and  $A_U$  and  $A_S$  are the absorbances of the assay solution and the Standard solution, respectively.

## Crotamiton Cream

» Crotamiton Cream contains not less than 93.0 percent and not more than 107.0 percent of the labeled amount of  $C_{13}H_{17}NO$ .

**Packaging and storage**—Preserve in collapsible tubes or tight, light-resistant containers.

### USP Reference standards (11)—

USP Crotamiton RS

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that of the *Standard preparation*, both relative to the internal standard, as obtained in the *Assay preparation*.

**Minimum fill** (755): meets the requirements.

### Assay—

**Internal standard solution**—Dissolve butyl benzoate in methanol to obtain a solution containing about 17.5 mg per mL.

**Mobile phase**—Prepare a suitable degassed and filtered mixture of acetonitrile and water (3:2).

**Standard solution**—Dissolve a suitable quantity of USP Crotamiton RS, accurately weighed, in methanol to obtain a solution having a known concentration of about 1 mg per mL.

**Standard preparation**—Pipet 10 mL of *Standard solution* and 5 mL of *Internal standard solution* into a 50-mL volumetric flask, dilute with methanol to volume, and mix.

**Assay preparation**—Transfer an accurately weighed portion of Crotamiton Cream, equivalent to about 50 mg of crotamiton, to a tared 50-mL volumetric flask. Add about 25 mL of methanol, and shake and sonicate to disperse the cream. Dilute with methanol to volume, and mix. Filter about 20 mL through moderately retentive filter paper. Pipet 10 mL of the clear filtrate and 5 mL of *Internal standard solution* into a 50-mL volumetric flask, dilute with methanol to volume, and mix.

**Procedure**—Inject equal volumes of the *Standard preparation* and the *Assay preparation* into a liquid chromatograph (see *Chromatography* (621)) equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm stainless steel column that contains packing L1. In a suitable chromatogram, the resolution, *R*, between peaks for crotamiton and butyl benzoate is not less than 3.0; and the lowest and highest peak response ratios (*R*<sub>S</sub>) of three replicate injections of the *Standard preparation* agree within 2.0%. Calculate the quantity, in mg, of  $C_{13}H_{17}NO$  in the portion of Cream taken by the formula:

$$250C(R_U / R_S)$$

in which *C* is the concentration, in mg per mL, of USP Crotamiton RS in the *Standard preparation*; and *R*<sub>U</sub> and *R*<sub>S</sub> are the peak response ratios of the crotamiton peak and the butyl benzoate peak obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cupric Chloride

$CuCl_2 \cdot 2H_2O$  170.48

$CuCl_2$  134.45

Copper chloride ( $CuCl_2$ ) dihydrate;

Copper(2+) chloride dihydrate [10125-13-0].

Anhydrous [7447-39-4].

### DEFINITION

Cupric Chloride contains NLT 99.0% and NMT 100.5% of  $CuCl_2$ , calculated on the dried basis.

### IDENTIFICATION

- **A. IDENTIFICATION TESTS—GENERAL, Chloride** (191): A solution (1 in 20) meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Copper** (191): A solution (1 in 20) meets the requirements.



**ASSAY****• PROCEDURE**

**Sample solution:** 8 mg/mL of Cupric Chloride in water

**Analysis:** To the *Sample solution* add 4 mL of acetic acid and 3 g of potassium iodide. Titrate the liberated iodine with 0.1 N sodium thiosulfate VS, adding 2 g of potassium thiocyanate and 3 mL of starch TS as the endpoint is approached. Each mL of 0.1 N sodium thiosulfate is equivalent to 13.45 mg of Cupric Chloride ( $\text{CuCl}_2$ ).

**Acceptance criteria:** 99.0%–100.5% on the dried basis

**IMPURITIES****• LIMIT OF SODIUM**

**Sample stock solution:** Transfer 10.0 g of Cupric Chloride to a 100-mL volumetric flask, add water, and swirl to dissolve. Add 5 mL of nitric acid, and dilute with water to volume. This solution contains 0.1 g/mL of cupric chloride.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*. Add 2 mL of potassium chloride solution (1 in 20) to each flask, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* (852)), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.

**Acceptance criteria:** NMT 0.02%

**Table 1**

Limit Test	Wave-length (nm)	Sample Weight (g)	Reference Ion Added (mg)	Flame Type	Background Correction
Sodium	589.0	0.1	0.01/0.02	Air-acetylene	No
Potassium	766.5	0.1	0.01/0.02	Air-acetylene	No
Calcium	422.7	2.0	0.05/0.10	Air-acetylene	No
Iron	248.3	2.0	0.05/0.10	Air-acetylene	Yes
Nickel	232.0	2.0	0.10/0.20	Air-acetylene	No

**• LIMIT OF POTASSIUM**

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* (852)), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.

**Acceptance criteria:** NMT 0.01%

**• LIMIT OF CALCIUM**

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* (852)), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.

**Acceptance criteria:** NMT 0.005%

**• LIMIT OF IRON**

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* (852)), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.

**Acceptance criteria:** NMT 0.005%

**• LIMIT OF NICKEL**

**Sample stock solution:** Prepare as directed in the test for *Limit of Sodium*.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of the *Sample stock solution* equivalent to the *Sample Weight* given in *Table 1*. To two of the flasks add the amounts of reference analyte ion specified in *Table 1*, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* (852)), analyze the *Sample solutions* by the method of standard addition analysis according to *Table 1*.

**Acceptance criteria:** NMT 0.01%

**• INSOLUBLE MATTER**

**Sample:** 10 g of Cupric Chloride

**Analysis:** Transfer the *Sample* to a 250-mL beaker. Add 100 mL of water and 2 mL of hydrochloric acid. Cover the beaker, and heat to boiling. Digest the hot solution on a steam bath for 1 h, and pass through a tared, filtering crucible of fine-pore size. Rinse the beaker with hot water, passing the rinsings through the filter, and finally wash the filter with additional hot water. Retain the combined filtrate and washings for the test for *Limit of Sulfate*. Dry the filter at 105°.

**Acceptance criteria:** The residue weighs NMT 1.0 mg (0.01%).

**• LIMIT OF SULFATE**

**Analysis:** Heat to boiling the combined filtrate and washings retained from the test for *Insoluble Matter*. Add 10 mL of barium chloride TS, digest for 2 h on a steam bath, and allow to stand overnight. Pass the solution through a tared, porcelain filtering crucible of medium-pore size, and wash the residue with two 10-mL portions of hot water. Ignite at  $800 \pm 25^\circ$  to constant weight.

**Acceptance criteria:** The weight of the residue, corrected for the weight obtained in a blank test, does not exceed 1.2 mg (0.005%).

**SPECIFIC TESTS**

- LOSS ON DRYING (731):** Dry a sample at 105° for 16 h: it loses 20.9%–21.4% of its weight.

**ADDITIONAL REQUIREMENTS**

- PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

**Cupric Chloride Injection****DEFINITION**

Cupric Chloride Injection is a sterile solution of Cupric Chloride in Water for Injection. It contains NLT 90.0% and NMT 110.0% of the labeled amount of copper (Cu).



**IDENTIFICATION**

- **A.** The *Sample solution* exhibits an absorption maximum at about 325 nm when prepared and tested as directed in the Assay.

**ASSAY**• **PROCEDURE**

**Sodium chloride solution:** 1.35 g/L of sodium chloride  
**Standard stock solution:** Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, and dilute with 0.2 N nitric acid to volume. This solution contains 1000 µg/mL of copper. Store in a polyethylene bottle.

**Standard solutions:** Pipet 15 mL of *Standard stock solution* into a 250-mL volumetric flask, dilute with water to volume, and mix. Transfer 4.0, 5.0, and 6.0 mL of this solution to separate 100-mL volumetric flasks containing 10 mL of *Sodium chloride solution*, dilute the contents of each flask with water to volume, and mix.

These *Standard solutions* contain 2.4, 3.0, and 3.6 µg of copper per mL, respectively.

**Sample stock solution:** Transfer a volume of *Injection*, equivalent to 2 mg of copper, into 100 mL of water.

**Sample solution:** Pipet 15 mL of the *Sample stock solution* into a 100-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the *Injection*, calculate the amount, in mg, of sodium chloride in the initial dilution, and add sufficient *Sodium chloride solution* to bring the total sodium content of this flask to 13.5 mg. Dilute with water to volume.

**Instrumental conditions**

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption

**Analytical wavelength:** 324.8 nm (copper emission line)

**Lamp:** Copper hollow-cathode

**Flame:** Air-acetylene

**Blank:** *Sodium chloride solution* and water (1 in 10)

**Analysis**

**Samples:** *Standard solutions* and *Sample solution*

Plot the absorbances of the *Standard solutions* versus concentration, in µg/mL, of copper, and draw the straight line best fitting the three plotted points.

From the graph so obtained, determine the concentration, *C*, in µg/mL, of copper in the *Sample solution*.

Calculate the percentage of copper in the portion of *Injection* taken:

$$\text{Result} = [(C/V) \times F \times V_1 \times D] \times (100/L)$$

*C* = concentration of copper in the *Sample solution* (µg/mL)

*V* = volume of *Injection* (mL)

*F* = conversion factor from µg to mg, 1/1000

*V*<sub>1</sub> = volume of the *Sample stock solution*, 100 mL

*D* = dilution factor from the *Sample solution*, 100/15

*L* = label claim (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

- **PH (791):** 1.5–2.5
- **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 250.0 USP Endotoxin Units/mg of copper.
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** Meets the requirements in *Injections and Implanted Drug Products* (1)

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

- **LABELING:** Label the *Injection* to indicate that it is to be diluted to the appropriate strength with Sterile Water for *Injection* or other suitable fluid before administration.
- **USP REFERENCE STANDARDS (11)**  
USP Endotoxin RS

**Cupric Sulfate**

CuSO<sub>4</sub> · 5H<sub>2</sub>O 249.69

CuSO<sub>4</sub> 159.61

Sulfuric acid, copper(2+) salt (1:1), pentahydrate;  
 Copper(2+) sulfate (1:1) pentahydrate [7758-99-8].  
 Anhydrous [7758-98-7].

**DEFINITION**

Cupric Sulfate is anhydrous or contains five molecules of water of hydration. It contains NLT 98.5% and NMT 100.5% of cupric sulfate (CuSO<sub>4</sub>), calculated on the dried basis.

**IDENTIFICATION**

- **A. IDENTIFICATION TESTS—GENERAL, Sulfate (191):** A 100-mg/mL solution meets the requirements.
- **B. IDENTIFICATION TESTS—GENERAL, Copper (191):** A 100-mg/mL solution meets the requirements.

**ASSAY**• **PROCEDURE**

**Sample solution:** Place 650 mg of Cupric Sulfate in a weighed container fitted with a ground-glass stopper. Dry, allow to cool in a desiccator, and weigh again to obtain the weight of the sample. Dissolve in 50 mL of water. Add 4 mL of 6 N acetic acid and 3 g of potassium iodide.

**Titrimetric system**

**Mode:** Direct titration

**Titrant:** 0.1 N sodium thiosulfate VS

**Endpoint detection:** Visual

**Analysis:** Titrate the liberated iodine in the *Sample solution* with the *Titrant*, adding about 2 g of potassium thiocyanate and 3 mL of starch TS as the endpoint is approached. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N sodium thiosulfate is equivalent to 15.96 mg of cupric sulfate (CuSO<sub>4</sub>).

**Acceptance criteria:** 98.5%–100.5% on the dried basis

**IMPURITIES**• **LIMIT OF SODIUM**

**Sample stock solution:** 0.2 g/mL of cupric sulfate in water, prepared as follows. Transfer 40.0 g of Cupric Sulfate to a 200-mL volumetric flask, add water, and swirl to dissolve. Add 5 mL of nitric acid, and dilute with water to volume.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in Table 1. To two of the flasks add the amounts of reference analyte ion specified in Table 1. Add 2 mL of potassium chloride solution (1 in 20) to each flask, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* (852)), analyze the *Sample solutions* by the method of standard addition analysis given in Table 1.



Table 1

Limit Test	Wave-length (nm)	Sample Weight (g)	Reference Ion Added (mg)	Flame Type	Back-ground Correction
Sodium	589.0	0.05	0.005/ 0.01	Air-acetylene	No
Potassium	766.5	0.4	0.02/ 0.04	Air-acetylene	No
Calcium	422.7	0.8	0.02/ 0.04	Nitrous oxide-acetylene	No
Iron	248.3	4.0	0.12/ 0.24	Air-acetylene	Yes
Nickel	232.0	4.0	0.10/ 0.20	Air-acetylene	No

Acceptance criteria: NMT 0.02%

• **LIMIT OF POTASSIUM**

**Sample stock solution:** Prepare as directed in the test for Limit of Sodium.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in Table 1. To two of the flasks add the amounts of reference analyte ion specified in Table 1, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* (852)), analyze the *Sample solutions* by the method of standard addition analysis given in Table 1.

Acceptance criteria: NMT 0.01%

• **LIMIT OF CALCIUM**

**Sample stock solution:** Prepare as directed in the test for Limit of Sodium.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in Table 1. To two of the flasks add the amounts of reference analyte ion specified in Table 1, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* (852)), analyze the *Sample solutions* by the method of standard addition analysis given in Table 1.

Acceptance criteria: NMT 0.005%

• **LIMIT OF IRON**

**Sample stock solution:** Prepare as directed in the test for Limit of Sodium.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in Table 1. To two of the flasks add the amounts of reference analyte ion specified in Table 1, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* (852)), analyze the *Sample solutions* by the method of standard addition analysis given in Table 1.

Acceptance criteria: NMT 0.003%

• **LIMIT OF NICKEL**

**Sample stock solution:** Prepare as directed in the test for Limit of Sodium.

**Sample solutions:** To three 25-mL volumetric flasks add a volume of *Sample stock solution* equivalent to the *Sample Weight* given in Table 1. To two of the flasks add the amounts of reference analyte ion specified in Table 1, and dilute with water to volume.

**Analysis:** Using atomic absorption spectrophotometry (see *Atomic Absorption Spectroscopy* (852)), analyze the *Sample solutions* by the method of standard addition analysis given in Table 1.

Acceptance criteria: NMT 0.005%

**SPECIFIC TESTS**

• **LOSS ON DRYING** (731)

**Analysis:** Dry it at 250° to constant weight.

Acceptance criteria: 33.0%–36.5% for the pentahydrate form; NMT 1.0% for the anhydrous form

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **LABELING:** Label the product to indicate whether it is anhydrous or it is the pentahydrate.

## Cupric Sulfate Injection

» Cupric Sulfate Injection is a sterile solution of Cupric Sulfate in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of copper (Cu).

**Packaging and storage—**Preserve in single-dose or multiple-dose containers, preferably of Type I or Type II glass.

**Labeling—**Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.

**USP Reference standards** (11)—

USP Endotoxin RS

**Identification—**The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 325 nm when tested as directed for *Procedure* in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 250.0 USP Endotoxin Units per mg of copper.

**pH** (791): between 2.0 and 3.5.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements—**It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay—**

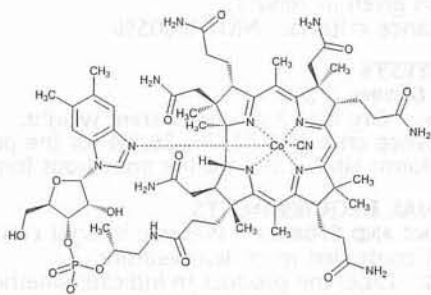
*Sodium chloride solution*, *Copper stock solution*, and *Standard preparations*—Prepare as directed in the *Assay* under *Cupric Chloride Injection*.

**Assay preparation—**Transfer an accurately measured volume of Injection, equivalent to about 2 mg of copper, to a 100-mL volumetric flask, dilute with water to volume, and mix. Pipet 15 mL of this solution into a 100-mL volumetric flask. From the labeled amount of sodium chloride, if any, in the Injection, calculate the amount, in mg, of sodium chloride in the initial dilution, and add sufficient *Sodium chloride solution* to bring the total sodium content of this flask to 13.5 mg. Dilute with water to volume, and mix.

**Procedure—**Proceed as directed for *Procedure* in the *Assay* under *Cupric Chloride Injection*.



## Cyanocobalamin



$C_{63}H_{88}CoN_{14}O_{14}P$   
Vitamin B<sub>12</sub> [68-19-9].

1355.37

### DEFINITION

Cyanocobalamin contains NLT 96.0% and NMT 102.0% of cyanocobalamin ( $C_{63}H_{88}CoN_{14}O_{14}P$ ), calculated on the dried basis.

### IDENTIFICATION

#### • A. ULTRAVIOLET ABSORPTION (197U)

Wavelength range: 200–700 nm

Sample solution: Prepare as directed in the Assay.

Acceptance criteria: The absorption spectrum exhibits maxima at  $278 \pm 1$ ,  $361 \pm 1$ , and  $550 \pm 2$  nm. The absorbance ratio  $A_{361}/A_{278}$  is 1.70–1.90, and the absorbance ratio  $A_{361}/A_{550}$  is 3.15–3.40.

#### • B.

Sample solution: Fuse 1 mg of Cyanocobalamin with 50 mg of potassium pyrosulfate in a porcelain crucible. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling.

Analysis: Add 1 drop of phenolphthalein TS, and add sodium hydroxide solution (100 mg/mL), dropwise, until just pink. Add 500 mg of sodium acetate, 0.5 mL of 1 N acetic acid, and 0.5 mL of nitroso R salt solution (2 mg/mL). Add 0.5 mL of hydrochloric acid, and boil for 1 min.

Acceptance criteria: A red or orange-red color appears immediately after the addition of nitroso R salt. The red color persists after boiling with the addition of hydrochloric acid.

#### • C. HPLC

Mobile phase and Chromatographic system: Proceed as directed in the test for Related Compounds.

Standard solution: 50 µg/mL of cyanocobalamin from USP Cyanocobalamin RS in Mobile phase. Use within 1 h.

Sample solution: 50 µg/mL of Cyanocobalamin in Mobile phase. Use within 1 h.

Acceptance criteria: The retention time of the major peak of the Sample solution corresponds to that of the Standard solution.

### ASSAY

#### Change to read:

#### • PROCEDURE

• (RB 1-Jun-2016)

Sample solution: 30 µg/mL of Cyanocobalamin in water

Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: UV

Analytical wavelength: 361 nm

Cell: 1 cm

Blank: Water

#### Analysis

• Sample: Sample solution

Calculate the percentage of cyanocobalamin ( $C_{63}H_{88}CoN_{14}O_{14}P$ ) in the portion of Cyanocobalamin taken:

$$\text{Result} = A_U / (A_S \times C_U)$$

$A_U$  = absorbance of the Sample solution

$A_S$  = specific absorbance ( $E_{1\%}^{1\text{cm}}$ ) of cyanocobalamin at 361 nm ( $100 \text{ mL} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$ ), 207

$C_U$  = concentration of Cyanocobalamin in the Sample solution (g/mL) • (RB 1-Jun-2016)

Acceptance criteria: 96.0%–102.0% on the dried basis

### IMPURITIES

#### • RELATED COMPOUNDS

Solution A: 10 g/L of disodium hydrogen phosphate in water

Mobile phase: Mixture of methanol and Solution A (26.5: 73.5). Adjust with phosphoric acid to a pH of 3.5.

System suitability solution: Dissolve 25 mg of Cyanocobalamin in 10 mL of water, warming if necessary. Allow to cool, add 5 mL of a 1.0-g/L solution of tosylchloramide sodium and 0.5 mL of 0.05 M hydrochloric acid, and then dilute with water to 25 mL. Shake and allow to stand for 5 min. Dilute 1.0 mL of this solution with Mobile phase to 10 mL, and inject immediately.

Quantitative limit solution: 1 µg/mL of Cyanocobalamin in Mobile phase. Use within 1 h.

Sample solution: 1 mg/mL of Cyanocobalamin in Mobile phase. Use within 1 h.

Chromatographic system  
(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 361 nm

Column: 4.6-mm × 25-cm; 5-µm packing L7

Column temperature: 35°

Flow rate: 0.8 mL/min

Injection volume: 20 µL

#### System suitability

Samples: System suitability solution and Quantitative limit solution

[NOTE—The System suitability solution should exhibit two major peaks, cyanocobalamin and 7β,8β-lactocyanocobalamin. The relative retention times for the two peaks are 1.0 and 1.2, respectively.]

#### Suitability requirements

Resolution: NLT 2.5 between cyanocobalamin and 7β,8β-lactocyanocobalamin, System suitability solution

Signal-to-noise ratio: NLT 5.0 for the major peak, Quantitative limit solution

#### Analysis

Sample: Sample solution

[NOTE—The run time should be at least three times the retention time of cyanocobalamin peak.]

Identify the impurities listed in Table 1, and measure the peak responses.

Calculate the percentage of individual impurities in the portion of Cyanocobalamin taken:

$$\text{Result} = (r_U/r_T) \times 100$$

$r_U$  = peak response of each impurity from the Sample solution

$r_T$  = sum of all the peak responses from the Sample solution

Acceptance criteria: See Table 1. [NOTE—Disregard any peak less than 0.1%.]



Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyanocobalamin	1.0	—
7β,8β-Lactocyanocobalamin	1.2	1.0
50-Carboxycyanocobalamin	1.4	0.5
34-Methylcyanocobalamin	1.5	2.0
32-Carboxycyanocobalamin	1.6	1.0
8-epi-Cyanocobalamin	2.5	1.0
Any other unidentified impurity	—	0.5
Total impurities	—	3.0

**SPECIFIC TESTS**• **LOSS ON DRYING** (731)

Sample: 25 mg

Analysis: Dry the *Sample* in a suitable vacuum drying apparatus at 105° and at a pressure of NMT 5 mm of mercury for 2 h.

Acceptance criteria: NMT 12.0%

**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store at controlled room temperature.• **USP REFERENCE STANDARDS** (11)

USP Cyanocobalamin RS

**Cyanocobalamin Injection**

» Cyanocobalamin Injection is a sterile solution of Cyanocobalamin in Water for Injection, or in Water for Injection rendered isotonic by the addition of Sodium Chloride. It contains not less than 95.0 percent and not more than 115.0 percent of the labeled amount of anhydrous cyanocobalamin ( $C_{63}H_{88}CoN_{14}O_{14}P$ ).

**Packaging and storage**—Preserve in light-resistant, single-dose or multiple-dose containers, preferably of Type I glass, and store at controlled room temperature.

**USP Reference standards** (11)—

USP Cyanocobalamin RS

USP Endotoxin RS

**Identification**—The absorption spectrum, in the range of 300 nm to 550 nm, of the solution employed for measurement of absorbance in the *Assay* exhibits maxima at the same wavelengths as that of a similar solution of USP Cyanocobalamin RS, concomitantly measured, and the ratio  $A_{361}/A_{550}$  is between 3.15 and 3.40.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.4 USP Endotoxin Unit per  $\mu\text{g}$  of cyanocobalamin.

**pH** (791): between 4.5 and 7.0.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—Dilute, if necessary, an accurately measured volume of Injection, equivalent to not less than 300  $\mu\text{g}$  of cyanocobalamin, quantitatively and stepwise with water to a concentration of about 30  $\mu\text{g}$  per mL. Dissolve an accurately weighed quantity of USP Cyanocobalamin RS in water, and dilute quantitatively and stepwise with water to obtain a Standard solution having a known concentration of about 30  $\mu\text{g}$  per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 361 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in

$\mu\text{g}$ , of  $C_{63}H_{88}CoN_{14}O_{14}P$  in each mL of the Injection taken by the formula:

$$10(C/V)(A_U / A_S)$$

in which C is the concentration, in  $\mu\text{g}$  per mL, of USP Cyanocobalamin RS in the Standard solution; V is the volume, in mL, of Injection taken; and  $A_U$  and  $A_S$  are the absorbances of the solution from the Injection and the Standard solution, respectively.

**Cyanocobalamin Tablets****DEFINITION**

Cyanocobalamin Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cyanocobalamin ( $C_{63}H_{88}CoN_{14}O_{14}P$ ).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay, Procedure 1* or *Procedure 2*.

**ASSAY**

[NOTE—Where more than one assay procedure is given in the monograph, the requirements may be met by following any one of the specified procedures. The procedure used is stated in the labeling only if *Procedure 1* is not used.]

• **PROCEDURE 1**

[NOTE—Use low-actinic glassware throughout this procedure.]

**Mobile phase:** Methanol and water (7:13)

**Standard solution:** 5  $\mu\text{g}/\text{mL}$  of cyanocobalamin from USP Cyanocobalamin RS in water

**Sample solution:** Finely powder NLT 30 Tablets. Transfer a portion of the powder, equivalent to 500  $\mu\text{g}$  of cyanocobalamin, to a 100-mL volumetric flask, add 60 mL of water, and sonicate for 5 min. Dilute with water to volume, and filter.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** 361 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu\text{m}$  packing L1

**Flow rate:** 0.5 mL/min

**Injection volume:** 100  $\mu\text{L}$

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cyanocobalamin ( $C_{63}H_{88}CoN_{14}O_{14}P$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of cyanocobalamin from USP Cyanocobalamin RS in the *Standard solution* ( $\mu\text{g}/\text{mL}$ )

$C_U$  = nominal concentration of cyanocobalamin in the *Sample solution* ( $\mu\text{g}/\text{mL}$ )

**Acceptance criteria:** 90.0%–110.0%

• **PROCEDURE 2**

[NOTE—Use low-actinic glassware throughout this procedure. Inject samples within 30 min.]

**Buffer:** Dissolve 470.5 mg of low UV hexanesulfonic acid sodium salt in water, add 1 mL of phosphoric acid,



dilute with water to 1000 mL, and mix. Adjust with 50% potassium hydroxide to a pH of 3.5.

**Mobile phase:** Acetonitrile and Buffer. See Table 1 for gradient.

Table 1

Time (min)	Acetonitrile (%)	Buffer (%)
0	1.0	99.0
0.5	1.0	99.0
1.2	2.3	97.7
1.4	5.0	95.0
2.5	7.0	93.0
5.0	18.0	82.0
5.5	25.0	75.0
6.5	25.0	75.0
7.0	1.0	99.0
8.0	1.0	99.0

**Standard solution:** 1 µg/mL of cyanocobalamin from USP Cyanocobalamin RS in water

**Sample solution:** Finely powder NLT 20 Tablets. Transfer a portion of the powder, equivalent to 25 µg of cyanocobalamin, to a suitable Erlenmeyer flask with a stopper, pipet 25 mL of water, sonicate for 5 min, and shake vigorously for 2 min. Pass through a membrane filter of 0.45-µm pore size.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** UPLC

**Detector:** UV 361 nm

**Column:** 2.1-mm × 10-cm; 1.7-µm packing L1

**Column temperature:** 35°

**Flow rate:** 0.5 mL/min

**Injection volume:** 15 µL

#### System suitability

**Sample:** Standard solution

**Suitability requirements**

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of cyanocobalamin (C<sub>63</sub>H<sub>88</sub>CoN<sub>14</sub>O<sub>14</sub>P) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of cyanocobalamin from USP Cyanocobalamin RS in the Standard solution (µg/mL)

$C_U$  = nominal concentration of cyanocobalamin in the Sample solution (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### • DISINTEGRATION (701)

**Medium:** Water

**Time:** 30 min. If the label recommends to disintegrate the Tablets in the mouth before swallowing: NMT 3 min

**Acceptance criteria:** Meet the requirements

##### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

#### ADDITIONAL REQUIREMENTS

##### • PACKAGING AND STORAGE:

Preserve in tight, light-resistant containers.

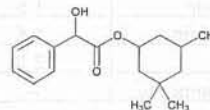
##### • LABELING:

Tablets that are intended to be disintegrated in the mouth before swallowing are so labeled. The labeling states with which assay procedure the product complies only if Procedure 1 is not used.

#### • USP REFERENCE STANDARDS (11)

USP Cyanocobalamin RS

### Cyclandelate



C<sub>17</sub>H<sub>24</sub>O<sub>3</sub> 276.37

3,3,5-Trimethylcyclohexanol α-phenyl-α-hydroxyacetate;  
1,5-cis-3,3,5-Trimethylcyclohexyl 2-hydroxy-2-phenyl acetate  
[456-59-7].

#### DEFINITION

Cyclandelate contains NLT 98.0% of cyclandelate (C<sub>17</sub>H<sub>24</sub>O<sub>3</sub>), calculated on the dried basis.

#### IDENTIFICATION

##### • A. ULTRAVIOLET ABSORPTION (197U)

**Sample solution:** 0.5 mg/mL of cyclandelate

**Medium:** Alcohol

**Acceptance criteria:** The solution exhibits absorption maxima between 250 and 254 nm, between 256 and 260 nm, and between 262 and 266 nm.

##### • B. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Sample solution:** 10 mg/mL of cyclandelate in alcohol

**Chromatographic system**

**Application volume:** 5 µL

**Developing solvent system:** Hexane, ethyl acetate, and glacial acetic acid (80:20:10)

**Acceptance criteria:** Meets the requirements

#### ASSAY

##### • PROCEDURE

**Mobile phase:** Acetonitrile and water (800:200)

**System suitability solution:** 0.2 mg/mL of USP Cyclandelate RS and 0.08 mg/mL of dicyclohexyl phthalate in Mobile phase

**Standard solution:** 0.2 mg/mL of USP Cyclandelate RS in Mobile phase

**Sample solution:** 0.2 mg/mL of Cyclandelate in Mobile phase

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 228 nm

**Column:** 4.0-mm × 15-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 10 µL

**System suitability**

**Samples:** System suitability solution and Standard solution

**Suitability requirements**

**Resolution:** NLT 7 between cyclandelate and dicyclohexyl phthalate, System suitability solution

**Relative standard deviation:** NMT 2.0% for replicate injections, Standard solution

#### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of cyclandelate (C<sub>17</sub>H<sub>24</sub>O<sub>3</sub>) in the portion of Cyclandelate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Cyclandelate RS in the Standard solution (mg/mL)



$C_U$  = concentration of Cyclandelate in the *Sample solution* (mg/mL)

Acceptance criteria: NLT 98.0% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

### Delete the following:

- **HEAVY METALS**, *Method II* (231): NMT 20 ppm (Official 1, Jan-2018)

### ORGANIC IMPURITIES

Mobile phase, System suitability solution, Standard solution, and System suitability: Proceed as directed in the *Assay*.

**Standard solution 1:** 0.03 mg/mL of cyclandelate in *Mobile phase* prepared as follows. Pipet 3.0 mL of the *Sample solution* into a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Sample solution:** 1 mg/mL of Cyclandelate in *Mobile phase*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Proceed as directed in the *Assay*, except for the following:

**Run time:** About 3 times the retention time of cyclandelate, *Sample solution*

### Analysis

**Samples:** *Standard solution 1* and *Sample solution*

**Acceptance criteria:** The total area of all the peaks from the *Sample solution*, other than the peak of cyclandelate, is NMT the peak area of cyclandelate from *Standard solution 1*.

**Total impurities:** NMT 3.0%

### SPECIFIC TESTS

- **LOSS ON DRYING** (731)

**Sample:** 1 g

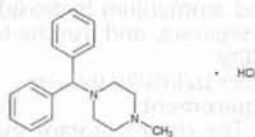
**Analysis:** Dry the *Sample* over silica gel for 24 h.

**Acceptance criteria:** NMT 0.5%

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store below 40°, preferably between 15° and 30°.
- **USP REFERENCE STANDARDS** (11)  
USP Cyclandelate RS

## Cyclizine Hydrochloride



$C_{18}H_{22}N_2 \cdot HCl$

302.84

Piperazine, 1-(diphenylmethyl)-4-methyl-, monohydrochloride;

1-(Diphenylmethyl)-4-methylpiperazine monohydrochloride [303-25-3].

### DEFINITION

Cyclizine Hydrochloride contains NLT 98.0% and NMT 100.5% of cyclizine hydrochloride ( $C_{18}H_{22}N_2 \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B. IDENTIFICATION TESTS—GENERAL**, *Chloride* (191): Meets the requirements

### ASSAY

#### PROCEDURE

[NOTE—In order to avoid overheating in the reaction medium, mix thoroughly throughout and stop the titration immediately after the endpoint has been reached.]

**Sample:** 120 mg

**Analysis:** Dissolve the *Sample* in 15 mL of anhydrous formic acid. Add 40 mL of acetic anhydride, and titrate with 0.1 N perchloric acid VS. Perform a blank determination (see *Titrimetry* (541)), and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 15.14 mg of cyclizine hydrochloride ( $C_{18}H_{22}N_2 \cdot HCl$ ).

Acceptance criteria: 98.0%–100.5% on the dried basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

#### ORGANIC IMPURITIES

[NOTE—Prepare solutions immediately before use.]

**Standard solution:** 0.05 mg/mL of USP Cyclizine Hydrochloride RS in methanol

**Impurity standard solution:** 0.25 mg/mL each of USP Cyclizine Hydrochloride RS, USP Cyclizine Related Compound A RS, and USP Benzhydrol RS in methanol

**Sample solution:** Prepare a solution containing 50 mg/mL of Cyclizine Hydrochloride by dissolving a suitable amount first in methanol, using 80% of the final volume, and then diluting with 1 N sodium hydroxide to volume.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.33-mm  $\times$  25-m; coated with a 0.5- $\mu$ m film of phase G27

### Temperatures

**Injection port:** 250°

**Detector:** 290°

**Column:** See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
100	10	240	0
240	15	270	14

**Carrier gas:** Helium

**Flow rate:** 1 mL/min

**Injection volume:** 1  $\mu$ L

**Injection type:** Split ratio, 1:25

### System suitability

**Sample:** *Impurity standard solution*

### Suitability requirements

**Peak-to-valley ratio:** NLT 50 between cyclizine related compound A and methanol

### Analysis

**Samples:** *Standard solution*, *Impurity standard solution*, and *Sample solution*

Calculate the percentage of cyclizine related compound A and benzhydrol in the portion of Cyclizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each related compound from the *Sample solution*

$r_S$  = peak response of the corresponding related compound from the *Impurity standard solution*

$C_S$  = concentration of the corresponding related compound in the *Impurity standard solution* (mg/mL)



$C_U$  = concentration of Cyclizine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any other individual impurity in the portion of Cyclizine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of cyclizine from the *Standard solution*

$C_S$  = concentration of USP Cyclizine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cyclizine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 2. Disregard any peak below 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
1-Methylpiperazine (cyclizine related compound A)	0.2	0.5
Benzhydrol	0.7	0.5
Cyclizine	1.0	—
Any other individual impurity	—	0.10
Total impurities	—	1.0

#### SPECIFIC TESTS

##### • PH (791)

**Sample solution:** 20 mg/mL in a mixture of alcohol and water (2:3)

**Acceptance criteria:** 4.5–5.5

##### • LOSS ON DRYING (731)

**Analysis:** Dry a sample at 120° for 3 h.

**Acceptance criteria:** NMT 1.0%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

##### • USP REFERENCE STANDARDS (11)

USP Cyclizine Hydrochloride RS

USP Cyclizine Related Compound A RS

1-Methylpiperazine.

$C_5H_{12}N_2$  100.16

USP Benzhydrol RS

Diphenylmethanol.

$C_{13}H_{12}O$  184.23

**Preparation and the Assay Preparation**, respectively, with an equal volume of dilute sulfuric acid (1 in 100), and determine the absorbance at the wavelength of maximum absorbance at about 264 nm.

Calculate the percentage of the labeled amount of cyclizine hydrochloride ( $C_{18}H_{22}N_2 \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Assay Preparation*

$A_S$  = absorbance of the *Standard Preparation*

$C_S$  = concentration of USP Cyclizine Hydrochloride RS in the *Standard Preparation* (mg/mL)

$C_U$  = nominal concentration of cyclizine hydrochloride in the *Assay Preparation* (mg/mL)

**Acceptance criteria:** 93.0%–107.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION, Procedure for a Pooled Sample (711)

**Medium:** Water; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Analysis:** Determine the amount of cyclizine hydrochloride ( $C_{18}H_{22}N_2 \cdot HCl$ ) dissolved by proceeding as directed in the *Assay*, making any necessary modifications.

**Tolerances:** NLT 75% (Q) of the labeled amount of cyclizine hydrochloride ( $C_{18}H_{22}N_2 \cdot HCl$ ) is dissolved.

##### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Diluent:** Methanol

**Standard solution 1:** 0.05 mg/mL of USP Cyclizine Hydrochloride RS in *Diluent*

**Standard solution 2:** 0.05 mg/mL of USP Cyclizine Related Compound A RS in *Diluent*

**System suitability solution:** 1 mg/mL of USP Cyclizine Hydrochloride RS and 1 mg/mL of USP Hydroxyzine Hydrochloride RS in *Diluent*

**Sample solution:** Triturate a quantity of powdered Tablets containing 100 mg of cyclizine hydrochloride with 10 mL of methanol, and filter.

##### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 20  $\mu$ L

**Developing solvent system:** Mix methylene chloride, methanol, and ammonium hydroxide (90:8:2). Allow the layers to separate, and use the lower layer.

##### System suitability

**Sample:** *System suitability solution*

##### Suitability requirements

**Resolution:** The chromatogram shows two clearly visible and separated spots.

##### Analysis

**Samples:** *Standard solution 1*, *Standard solution 2*, and *Sample solution*

Proceed as directed in *Chromatography* (621), *Thin-Layer Chromatography*. Air-dry the plate for several min, expose it to iodine vapor for 20 min, and examine the plate under short-wavelength UV light.

##### Acceptance criteria

**Cyclizine related compound A:** The spot corresponding to cyclizine related compound A in the *Sample solution* is not more intense than the principal spot obtained from *Standard solution 2* (NMT 0.5%).

**Any unspecified impurity:** Any other secondary spot in the chromatogram from the *Sample solution* is not

## Cyclizine Hydrochloride Tablets

#### DEFINITION

Cyclizine Hydrochloride Tablets contain NLT 93.0% and NMT 107.0% of the labeled amount of cyclizine hydrochloride ( $C_{18}H_{22}N_2 \cdot HCl$ ).

#### IDENTIFICATION

##### • A. INFRARED ABSORPTION (197K)

**Sample:** Extract a quantity of powdered Tablets containing 100 mg of cyclizine hydrochloride with 10 mL of ethanol. Filter, evaporate to dryness, and use the dried residue.

**Acceptance criteria:** Meet the requirements

#### ASSAY

##### • PROCEDURE

**Analysis:** Proceed with Tablets as directed in *Salts of Organic Nitrogenous Bases* (501). Dilute the *Standard*

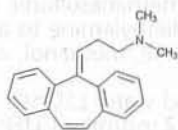


more intense than the principal spot obtained from *Standard solution 1* (NMT 0.5%).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**
  - USP Cyclizine Hydrochloride RS
  - USP Cyclizine Related Compound A RS
  - 1-Methylpiperazine.
  - C<sub>5</sub>H<sub>12</sub>N<sub>2</sub> 100.16
  - USP Hydroxyzine Hydrochloride RS

### Cyclobenzaprine Hydrochloride



C<sub>20</sub>H<sub>21</sub>N · HCl 311.85  
1-Propanamine, 3-(5H-dibenzo[*a,d*]cyclohepten-5-ylidene)-*N,N*-dimethyl-, hydrochloride;  
*N,N*-Dimethyl-5H-dibenzo[*a,d*]cycloheptene-Δ<sup>5,7</sup>-propylamine hydrochloride [6202-23-9].

#### DEFINITION

Cyclobenzaprine Hydrochloride contains NLT 98.0% and NMT 102.0% of cyclobenzaprine hydrochloride (C<sub>20</sub>H<sub>21</sub>N · HCl), calculated on the dried basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride (191):** A 20-mg/mL solution meets the requirements.

#### ASSAY

##### PROCEDURE

**Mobile phase:** Dissolve 2.0 g of ammonium acetate in 350 mL of water. Add 650 mL of methanol, and adjust with 25% ammonium hydroxide to a pH of 8.9.

**Standard solution:** 0.2 mg/mL of USP Cyclobenzaprine Hydrochloride RS in *Mobile phase*

**Sample solution:** 0.2 mg/mL of Cyclobenzaprine Hydrochloride in *Mobile phase*

**Chromatographic system**  
(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 226 nm

**Column:** 4.6-mm × 15 cm; 5-μm packing L1

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**Run time:** About 2 times the retention time of cyclobenzaprine

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cyclobenzaprine hydrochloride (C<sub>20</sub>H<sub>21</sub>N · HCl) in the portion of Cyclobenzaprine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response from the *Sample solution*  
 $r_s$  = peak response from the *Standard solution*  
 $C_s$  = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_u$  = concentration of Cyclobenzaprine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

#### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 10 ppm (Official 1, Jan-2018)

#### ORGANIC IMPURITIES

**Mobile phase:** Dissolve 4.0 g of ammonium acetate in 350 mL of water. Add 650 mL of methanol, and adjust with diluted acetic acid or diluted ammonia to a pH of 7.2.

**System suitability solution:** 0.4 mg/mL of USP Cyclobenzaprine Hydrochloride RS and 0.6 μg/mL each of USP Cyclobenzaprine Related Compound A RS and USP Cyclobenzaprine Related Compound B RS in *Mobile phase*

**Sample solution:** 0.4 mg/mL of Cyclobenzaprine Hydrochloride in *Mobile phase*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 226 nm

**Column:** 4.6-mm × 25 cm; 5-μm packing L7

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**Run time:** About 3 times the retention time of cyclobenzaprine

**System suitability**

**Sample:** *System suitability solution*

[NOTE—See *Table 1* for relative retention times.]

**Suitability requirements**

**Resolution:** NLT 2.0 between the cyclobenzaprine related compound A and cyclobenzaprine related compound B peaks

**Tailing factor:** NMT 2.0 for the cyclobenzaprine peak

**Relative standard deviation:** NMT 2.0% for cyclobenzaprine

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each specified impurity and any individual unspecified impurity in the portion of Cyclobenzaprine Hydrochloride taken:

$$\text{Result} = (r_u/r_T) \times (1/F) \times 100$$

- $r_u$  = peak response of each impurity from the *Sample solution*  
 $r_T$  = sum of the responses for all peaks from the *Sample solution*  
 $F$  = relative response factor for each impurity (see *Table 1*)

**Acceptance criteria:** See *Table 1*.



Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (% w/w)
Cyclobenzaprine related compound A	0.51	0.66	0.15
Cyclobenzaprine related compound B	0.59	1.0	0.15
Cyclobenzaprine N-oxide <sup>a</sup>	0.74	0.93	0.15
Cyclobenzaprine	1.0	—	—
Amitriptyline <sup>b</sup>	1.29	0.36	0.15
Dibenzocycloheptenone <sup>c</sup>	1.59	0.64	0.15
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

<sup>a</sup> 3-(5*H*-Dibenzo[*a,d*]cyclohepten-5-ylidene)-*N,N*-dimethyl-1-propanamine N-oxide.

<sup>b</sup> 10,11-Dihydro-*N,N*-dimethyl-5*H*-dibenzo[*a,d*]cycloheptene- $\Delta^5,7$ -propylamine.

<sup>c</sup> Dibenzof[*a,d*]cyclohepten-5-one.

### SPECIFIC TESTS

#### • LOSS ON DRYING (731)

Analysis: Dry a sample at 105° to constant weight.

Acceptance criteria: NMT 1.0%

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in well-closed containers.

#### • USP REFERENCE STANDARDS (11)

USP Cyclobenzaprine Hydrochloride RS

USP Cyclobenzaprine Related Compound A RS

5-[3-(Dimethylamino)propyl]-5*H*-dibenzo[*a,d*]cyclohepten-5-ol.

C<sub>20</sub>H<sub>23</sub>NO 293.40

USP Cyclobenzaprine Related Compound B RS

3-(5*H*-Dibenzo[*a,d*]cyclohepten-5-ylidene)-*N*-methyl-1-propanamine.

C<sub>19</sub>H<sub>19</sub>N 261.36

## Cyclobenzaprine Hydrochloride Extended-Release Capsules

### DEFINITION

Cyclobenzaprine Hydrochloride Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of cyclobenzaprine hydrochloride (C<sub>20</sub>H<sub>21</sub>N · HCl).

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197A)

**Standard:** Transfer 90 mg of USP Cyclobenzaprine Hydrochloride RS to a 25-mL volumetric flask. Add 10 mL of water and sonicate for 30 s. Transfer the solution to a 40-mL vial. Add 10 mL of methylene chloride. Cap the vial and shake. Centrifuge, if necessary, and transfer the lower methylene chloride layer to a watch glass. Evaporate the solvent, and use the residue. [NOTE—The use of a glass vial and a centrifuge speed of NMT 2000 rpm may be suitable. An infrared heat lamp may be used to evaporate the solvent.]

**Sample:** Transfer a portion of the contents of NLT 10 Capsules containing nominally 90 mg of cyclobenzaprine hydrochloride to a 25-mL volumetric flask. Add 10 mL of water and sonicate for 30 s. Trans-

fer the solution to a 40-mL vial. Add 10 mL of methylene chloride. Cap the vial and shake. Centrifuge, and transfer the lower methylene chloride layer to a watch glass. Evaporate the solvent, and use the residue.

[NOTE—The use of a glass vial and a centrifuge speed of NMT 2000 rpm may be suitable. An infrared heat lamp may be used to evaporate the solvent.]

### Analysis

**Samples:** *Standard* and *Sample*

**Acceptance criteria:** The maxima of the IR spectrum from the *Sample* correspond to those from the *Standard*.

- **B.** The retention time of the major peak of the *Sample* solution corresponds to that of the *Standard* solution, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** Add 3.7 mL of methanesulfonic acid per 1 L of water and adjust with diethylamine to a pH of 3.6.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (25.5: 21.0: 53.5)

**Diluent:** Acetonitrile and water (50:50)

**Standard solution:** 0.12 mg/mL of USP

Cyclobenzaprine Hydrochloride RS in *Diluent*

**Sample solution:** Nominally 0.12 mg/mL of cyclobenzaprine hydrochloride from Capsules prepared as follows. Remove the contents of NLT 20 Capsules, and transfer a suitable portion of the contents to a volumetric flask. Add 80% of the final flask volume of *Diluent*. Stir and sonicate, if necessary. Dilute with *Diluent* to volume, and centrifuge. Use the supernatant.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 290 nm

**Column:** 4.6-mm × 15-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

#### System suitability

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 1.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cyclobenzaprine hydrochloride (C<sub>20</sub>H<sub>21</sub>N · HCl) in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

*r<sub>U</sub>* = peak response from the *Sample solution*

*r<sub>S</sub>* = peak response from the *Standard solution*

*C<sub>S</sub>* = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)

*C<sub>U</sub>* = nominal concentration of cyclobenzaprine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm, wire helix sinkers

**Times:** 2, 4, 8, and 16 h

**Solution A:** Transfer 10 empty Capsule shells to a 1-L volumetric flask. Add 80% of the final flask volume of *Medium*, which has been warmed to 37°, and stir until the Capsule shells have dissolved. Allow to cool, and dilute with *Medium* to volume.

**Standard solution:** (L/900) mg/mL of USP

Cyclobenzaprine Hydrochloride RS in *Medium*, where *L*



is the label claim of cyclobenzaprine hydrochloride in mg/Capsule

**Sample solution:** Centrifuge a portion of the solution under test. Use the supernatant.

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** 290 nm

**Cell:** 1 cm

**Blank:** A mixture of *Solution A* and *Medium* prepared as follows. Transfer 10% of the final flask volume of *Solution A* to a suitable volumetric flask. Dilute with *Medium* to volume, centrifuge, and use the supernatant.

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank*. Correct the instrument by using the *Blank*.

Calculate the concentration ( $C_i$ ) of cyclobenzaprine hydrochloride ( $C_{20}H_{21}N \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_i = (A_U/A_S) \times C_S$$

$A_U$  = absorbance from the *Sample solution* at time point  $i$

$A_S$  = absorbance from the *Standard solution*

$C_S$  = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)

Calculate the percentage of the labeled amount of cyclobenzaprine hydrochloride ( $C_{20}H_{21}N \cdot HCl$ ) dissolved at each time point ( $i$ ):

$$\text{Result}_1 = C_i \times V \times (1/L) \times 100$$

$$\text{Result}_2 = \{[C_2 \times (V - V_3)] + (C_1 \times V_3)\} \times (1/L) \times 100$$

$$\text{Result}_3 = \{[C_3 \times (V - (2 \times V_3))] + [(C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{[C_4 \times (V - (3 \times V_3))] + [(C_3 + C_2 + C_1) \times V_3]\} \times (1/L) \times 100$$

$C_i$  = concentration of cyclobenzaprine hydrochloride in the portion of sample withdrawn at the specified time point (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Capsule)

$V_3$  = volume of the *Sample solution* withdrawn at each time point (mL)

**Tolerances:** See *Table 1*.

**Table 1**

Time Point (i)	Time (h)	Amount Dissolved (%)
1	2	NMT 40
2	4	43–63
3	8	66–86
4	16	NLT 80

The percentage of the labeled amount of cyclobenzaprine hydrochloride ( $C_{20}H_{21}N \cdot HCl$ ) dissolved at the times specified conform to *Acceptance Table 2* in (711).

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

**Buffer:** Dissolve 10 mL of *n*-butylamine in 900 mL of water, and adjust with acetic acid to a pH of 6.0. Dilute to 1 L.

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (15:29:56)

**Sensitivity solution:** 0.001 mg/mL of USP

Cyclobenzaprine Hydrochloride RS in *Mobile phase*

**Standard solution:** 0.02 mg/mL of USP

Cyclobenzaprine Hydrochloride RS and 0.01 mg/mL of USP Amitriptyline Hydrochloride RS in *Mobile phase*

**Sample solution:** Nominally 2 mg/mL of cyclobenzaprine hydrochloride from Capsules prepared as follows. Remove the contents of NLT 20 Capsules, and transfer a suitable portion of the contents to a volumetric flask. Add 70% of the final flask volume of *Mobile phase*. Stir and sonicate, if necessary. Dilute with *Mobile phase* to volume, and centrifuge. Use the supernatant.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 239 nm

**Column:** 4.6-mm  $\times$  15-cm; 5- $\mu$ m packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 20  $\mu$ L

**Run time:** NLT 3.5 times the retention time of cyclobenzaprine

**System suitability**

**Samples:** *Standard solution* and *Sensitivity solution*

[NOTE—The relative retention times for cyclobenzaprine and amitriptyline are 1.0 and 1.3, respectively. For other relative retention times, see *Table 2*.]

**Suitability requirements**

**Resolution:** NLT 2.5 between cyclobenzaprine and amitriptyline, *Standard solution*

**Relative standard deviation:** NMT 2.0% for cyclobenzaprine, *Standard solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each unspecified degradation product in the portion of Capsules taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each unspecified degradation product from the *Sample solution*

$r_S$  = peak response of cyclobenzaprine from the *Standard solution*

$C_S$  = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cyclobenzaprine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** See *Table 2*. Disregard peaks less than 0.05%.

**Table 2**

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyclobenzaprine related compound A <sup>a,b</sup>	0.5	—
Cyclobenzaprine	1.0	—

<sup>a</sup> 5-[3-(Dimethylamino)propyl]-5H-dibenzo[a,d]-cyclohepten-5-ol.

<sup>b</sup> This is a process impurity that is included in the table for identification purposes only. It is controlled in the drug substance and is not to be reported or included in the total impurities for the drug product.

<sup>c</sup> Dibenzo[a,d]cycloheptene-5-one (also known as dibenzocycloheptenone).



Table 2 (Continued)

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyproheptadine related compound B <sup>b,c</sup>	2.9	—
Any individual unspecified degradation product	—	0.2
Total degradation products	—	0.5

<sup>a</sup> 5-[3-(Dimethylamino)propyl]-5H-dibenzo[a,d]-cyclohepten-5-ol.

<sup>b</sup> This is a process impurity that is included in the table for identification purposes only. It is controlled in the drug substance and is not to be reported or included in the total impurities for the drug product.

<sup>c</sup> Dibenzo[a,d]cycloheptene-5-one (also known as dibenzocycloheptenone).

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
USP Amitriptyline Hydrochloride RS  
USP Cyclobenzaprine Hydrochloride RS

### Cyclobenzaprine Hydrochloride Tablets

#### DEFINITION

Cyclobenzaprine Hydrochloride Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of cyclobenzaprine hydrochloride ( $C_{20}H_{21}N \cdot HCl$ ).

#### IDENTIFICATION

##### A. INFRARED ABSORPTION (197M)

**Sample:** Transfer an amount equivalent to 50 mg of cyclobenzaprine hydrochloride from a quantity of finely powdered Tablets to a small flask. Add 10 mL of methylene chloride, swirl to dissolve, and filter. Evaporate the clear filtrate to about 5 mL, transfer to a centrifuge tube, and add 1–2 mL of ether. Evaporate with the aid of a current of air to about 1 mL, and agitate until crystallization occurs. Wash the crystals with several portions of ether, and air-dry.

**Acceptance criteria:** Meet the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

**Buffer:** 11.4 g/L of ammonium acetate in water. Adjust with ammonium hydroxide to a pH of 7.2.

**Mobile phase:** Methanol and *Buffer* (65:35)

**Standard solution:** 0.2 mg/mL of USP Cyclobenzaprine Hydrochloride RS in *Mobile phase*. Sonication may be used to aid in dissolution.

**Sample solution:** Nominally 0.2 mg/mL of cyclobenzaprine hydrochloride from NLT 20 finely powdered Tablets in *Mobile phase* prepared as follows.

Transfer a suitable amount of the powder to a suitable volumetric flask. Add 60% of the flask volume of *Mobile phase*, and sonicate for 30 min. Allow the solution to cool to room temperature, and then dilute with *Mobile phase* to volume. Centrifuge the solution, and use the supernatant.

**Chromatographic system**

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** UV 226 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 0.85%

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cyclobenzaprine hydrochloride ( $C_{20}H_{21}N \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cyclobenzaprine hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

##### DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 1:** 50 rpm

**Time:** 30 min

**Sample solution:** Pass a portion of the solution under test through a suitable filter, and dilute with *Medium* if necessary.

**Standard solution:** USP Cyclobenzaprine Hydrochloride RS in *Medium* with a concentration similar to the one expected in the *Sample solution*

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* <857>.)

**Mode:** UV

**Analytical wavelength:** 290 nm

**Analysis**

**Samples:** *Sample solution* and *Standard solution*

Calculate the percentage of the labeled amount of cyclobenzaprine hydrochloride ( $C_{20}H_{21}N \cdot HCl$ ) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of cyclobenzaprine hydrochloride ( $C_{20}H_{21}N \cdot HCl$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

#### IMPURITIES

##### ORGANIC IMPURITIES

**Buffer and Mobile phase:** Proceed as directed in the *Assay*.

**Standard solution:** 0.6 μg/mL each of USP

Cyclobenzaprine Hydrochloride RS, USP

Cyclobenzaprine Related Compound A RS, and USP

Cyclobenzaprine Related Compound B RS in *Mobile phase*

**Sample solution:** Nominally 400 μg/mL of cyclobenzaprine hydrochloride from NLT 20 finely powdered Tablets



dered Tablets in *Mobile phase* prepared as follows. Transfer a suitable amount of the powder to a suitable volumetric flask. Add 75% of the flask volume of *Mobile phase*, and sonicate for 30 min. Allow the solution to cool to room temperature, and then dilute with *Mobile phase* to volume. Centrifuge the solution, and use the supernatant.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 226 nm

Column: 4.6-mm × 25-cm; 5-μm packing L7

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 10 μL

Run time: NLT 3 times the retention time of cyclobenzaprine

#### System suitability

Sample: Standard solution

[NOTE—See Table 1 for relative retention times.]

#### Suitability requirements

Resolution: NLT 2.0 between the cyclobenzaprine related compound A and cyclobenzaprine related compound B peaks

Relative standard deviation: NMT 2.0% for the cyclobenzaprine peak

#### Analysis

Sample: Standard solution and Sample solution

Calculate the percentage of any individual degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any individual degradation product from the *Sample solution*

$r_S$  = peak response of cyclobenzaprine from the *Standard solution*

$C_S$  = concentration of USP Cyclobenzaprine Hydrochloride RS in the *Standard solution* (μg/mL)

$C_U$  = nominal concentration of cyclobenzaprine hydrochloride in the *Sample solution* (μg/mL)

Acceptance criteria: See Table 1.

Table 1

Name	Relative Retention Time	Acceptance Criteria NMT (%)
Cyclobenzaprine related compound A <sup>a</sup>	0.51	—
Cyclobenzaprine related compound B <sup>a</sup>	0.59	—
Cyclobenzaprine N-oxide <sup>b</sup>	0.74	0.2
Cyclobenzaprine	1.0	—
Amitriptyline <sup>b,c</sup>	1.3	—
Dibenzocycloheptene <sup>a,d</sup>	1.6	—
Any individual unspecified degradation product	—	0.1
Total degradation products	—	2.0

<sup>a</sup> Process impurity included for identification only and not included in the calculation of total degradation products.

<sup>b</sup> 3-(5*H*-Dibenzo[*a,d*]cyclohepten-5-ylidene)-*N,N*-dimethyl-1-propanamine N-oxide.

<sup>c</sup> 10,11-Dihydro-*N,N*-dimethyl-5*H*-dibenzo[*a,d*]cycloheptene-Δ<sup>2,7</sup>-proplylamine.

<sup>d</sup> Dibenzo[*a,d*]cyclohepten-5-one.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

#### • USP REFERENCE STANDARDS (11)

USP Cyclobenzaprine Hydrochloride RS

USP Cyclobenzaprine Related Compound A RS  
5-[3-(Dimethylamino)propyl]-5*H*-dibenzo[*a,d*]cyclohepten-5-ol.

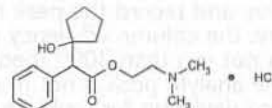
C<sub>20</sub>H<sub>23</sub>NO 293.40

USP Cyclobenzaprine Related Compound B RS

3-(5*H*-Dibenzo[*a,d*]cyclohepten-5-ylidene)-*N*-methyl-1-propanamine hydrochloride.

C<sub>19</sub>H<sub>19</sub>N · HCl 297.82

### Cyclopentolate Hydrochloride



C<sub>17</sub>H<sub>25</sub>NO<sub>3</sub> · HCl 327.85

Benzeneacetic acid, α-(1-hydroxycyclopentyl)-, 2-(dimethylamino)ethyl ester, hydrochloride, (±)-;

2-(Dimethylamino)ethyl (±)-1-hydroxy-α-phenylcyclopentaneacetate hydrochloride [5870-29-1].

» Cyclopentolate Hydrochloride contains not less than 98.0 percent and not more than 102.0 percent of C<sub>17</sub>H<sub>25</sub>NO<sub>3</sub> · HCl, calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers, and store in a cold place.

**USP Reference standards** (11)—  
USP Cyclopentolate Hydrochloride RS

#### Identification—

A: Infrared Absorption (197K).

B: A solution (1 in 500) responds to the tests for Chloride (191).

pH (791): between 4.5 and 5.5, in a solution (1 in 100).

**Loss on drying** (731)—Dry it at 105° for 4 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.05%.

#### Chromatographic purity—

*Buffer solution*, *Mobile phase*, and *Chromatographic system*—Prepare as directed under Assay.

*Test preparation*—Use the Assay preparation.

*Procedure*—Inject a volume (about 20 μL) of the *Test preparation* into the chromatograph, record the chromatogram obtained for a period of not less than twice the retention time of cyclopentolate, and measure the peak responses. Calculate the percentage of each peak, other than the solvent peak and the cyclopentolate peak, in the specimen of Cyclopentolate Hydrochloride taken by the same formula:

$$100r_i/r_t$$

in which  $r_i$  is the response of each peak and  $r_t$  is the sum of the responses of all of the peaks, excluding that of the solvent peak: not more than 1.0% individual impurity and not more than 2.0% total impurities are found.

#### Assay—

*Buffer solution*—Dissolve 660 mg of dibasic ammonium phosphate in 1000 mL of water. Adjust with phosphoric acid to a pH of 3.0 ± 0.1, and mix.

*Mobile phase*—Prepare a suitable filtered and degassed mixture of acetonitrile and *Buffer solution* (7:3). Make adjust-



ments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyclopentolate Hydrochloride RS in water, dilute quantitatively, and stepwise if necessary, with water, and mix to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Transfer about 100 mg of Cyclopentolate Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with water to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 220-nm detector and a 4.6-mm × 15-cm column that contains packing L15. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 3000 theoretical plates, the tailing factor for the analyte peak is not more than 2.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{17}H_{25}NO_3 \cdot HCl$  in the portion of Cyclopentolate Hydrochloride taken by the formula:

$$1000C(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Cyclopentolate Hydrochloride RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the cyclopentolate peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cyclopentolate Hydrochloride Ophthalmic Solution

» Cyclopentolate Hydrochloride Ophthalmic Solution is a sterile, aqueous solution of Cyclopentolate Hydrochloride. It may contain suitable buffers and other additives. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{17}H_{25}NO_3 \cdot HCl$ .

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**USP Reference standards** (11)—  
USP Cyclopentolate Hydrochloride RS

**Identification**—Place in a 125-mL separator a volume of Ophthalmic Solution, equivalent to about 50 mg of cyclopentolate hydrochloride, and place in a second separator about 50 mg of USP Cyclopentolate Hydrochloride RS dissolved in 5 mL of water. Treat each solution as follows. Add 1 g of potassium carbonate, and extract with two 10-mL portions of ether. Pass the ether extracts through ether-washed filter paper, collect the filtrate in a small beaker, and evaporate to dryness: the residue so obtained responds to *Identification test A* under *Cyclopentolate Hydrochloride*.

**Sterility Tests** (71): meets the requirements.

**pH** (791): between 3.0 and 5.5.

**Assay**—

*Buffer solution, Mobile phase, Standard preparation, and Chromatographic system*—Proceed as directed in the *Assay* under *Cyclopentolate Hydrochloride*.

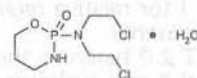
**Assay preparation**—Transfer an accurately measured volume of Ophthalmic Solution, equivalent to about 10 mg of cyclopentolate hydrochloride, to a 100-mL volumetric flask, dilute with water to volume, and mix.

**Procedure**—Proceed as directed in the *Assay* under *Cyclopentolate Hydrochloride*. Calculate the quantity, in mg, of cyclopentolate hydrochloride ( $C_{17}H_{25}NO_3 \cdot HCl$ ) in each mL of the Ophthalmic Solution taken by the formula:

$$100(C / V)(r_u / r_s)$$

in which  $V$  is the volume, in mL, of Ophthalmic Solution taken, and the other terms are as defined therein.

## Cyclophosphamide



$C_7H_{15}Cl_2N_2O_2P \cdot H_2O$  279.10

$C_7H_{15}Cl_2N_2O_2P$  261.09

2H-1,3,2-Oxazaphosphorin-2-amine, *N,N*-bis(2-chloroethyl)tetrahydro-, 2-oxide, monohydrate, (±); (±)-2-[Bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate [6055-19-2]. Anhydrous [50-18-0].

### DEFINITION

Cyclophosphamide contains NLT 97.0% and NMT 103.0% of  $C_7H_{15}Cl_2N_2O_2P$ , calculated on the anhydrous basis.

[CAUTION—Great care should be taken in handling Cyclophosphamide, as it is a potent cytotoxic agent.]

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** Acetonitrile and water (3:7)

**Ethylparaben solution:** Dissolve 185 mg of ethylparaben in 250 mL of alcohol in a 1000-mL volumetric flask, and dilute with water to volume.

**System suitability solution:** Transfer USP Cyclophosphamide RS, equivalent to 25 mg of anhydrous cyclophosphamide, to a 50-mL volumetric flask, add 25 mL of water, and shake to dissolve the USP Reference Standard. Add 5.0 mL of *Ethylparaben solution*, and dilute with water to volume.

**Standard solution:** 0.5 mg/mL of USP Cyclophosphamide RS in water

**Sample solution:** 0.5 mg/mL of Cyclophosphamide in water

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 195 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection size:** 25 µL

#### System suitability

**Sample:** *System suitability solution*

[NOTE—The relative retention times for cyclophosphamide and ethylparaben are about 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2 between cyclophosphamide and ethylparaben



**Relative standard deviation:** NMT 2% from six replicate injections, cyclophosphamide peak

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of  $C_7H_{15}Cl_2N_2O_2P$  in the Cyclophosphamide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cyclophosphamide RS in the *Standard solution* (mg/mL). [NOTE—Concentration is calculated on the anhydrous basis.]

$C_U$  = concentration of Cyclophosphamide in the *Sample solution* (mg/mL). [NOTE—Nominal concentration is calculated on the anhydrous basis.]

**Acceptance criteria:** 97.0%–103.0% on the anhydrous basis

#### IMPURITIES

##### Inorganic Impurities

##### Delete the following:

- **HEAVY METALS (231):** NMT 20 ppm  
*Sample solution:* 40 mg/mL, and filter if necessary. (Official 1-Jan-2018)

##### Organic Impurities

##### • PROCEDURE 1: LIMIT OF PROPANOLAMINE

**Diluent:** Methylene chloride and dehydrated alcohol (17:3)

**Standard solution:** 12.5 µg/mL of USP Propanolamine RS in *Diluent*. [NOTE—Propanolamine in the *Standard solution* is 0.025% of Cyclophosphamide in the *Sample solution*.]

**Sample solution:** 50 mg/mL of Cyclophosphamide in *Diluent*

##### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.1-mm layer of chromatographic silica gel

**Application volume:** 2 µL

**Developing solvent system A:** Toluene, methylene chloride, and methanol (5:5:1). Prepare at time of use.

**Developing solvent system B:** Methanol and glacial acetic acid (9:1)

**Solution A:** Hydrochloric acid and water (7:18)

**Solution B:** 5 g/L of potassium permanganate in water

**Reagent A:** *Solution A* and *Solution B* (1:1). [NOTE—Mix in a small beaker at the time of use under a fume hood to generate chlorine gas, and immediately place the beaker with solution into closed TLC chamber (placed in a fume hood).]

**Reagent B:** 100 mg of tetramethylbenzidine in 2.5 mL of methylene chloride, and dilute with cyclohexane to 100 mL

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Develop with *Developing solvent system A* over a path of 7 cm followed by air drying for 15 min. Develop again in *Developing solvent system B* over a path of 2 cm followed by air drying for NLT 10 min. [NOTE—Transfer *Developing solvent system B* to the chamber 15 min before development.] Dry the plate at 45° under a vacuum for 50 min. Place the plate in a closed chromatography tank (placed in a fume hood) containing *Reagent A*, and leave the plate in the tank

for 10 min. Remove the plate and place it in a fume hood for 10 min to remove the excess chlorine. Stain the plate by dipping it into *Reagent B*. Remove it from *Reagent B* and wait for 15 min, evaluate it with a suitable densitometer, equipped with a filter having its maximum transmittance at 375 nm, and locate and scan the spot produced by propanolamine from the *Standard solution* and any spot from the *Sample solution* having the same  $R_f$  as that produced by propanolamine from the *Standard solution*.

##### Acceptance criteria

**Propanolamine:** The spot of propanolamine from the *Sample solution* is not more intense than the spot of propanolamine from the *Standard solution* (0.025%).

##### • PROCEDURE 2: LIMIT OF DEGRADATION PRODUCTS

**Diluent:** Methanol and water (1:1)

**Standard solution A:** 12 µg/mL of USP Cyclophosphamide Related Compound A RS in *Diluent*

**Standard solution B:** 12 µg/mL of USP Cyclophosphamide Related Compound B RS in *Diluent*

**Standard solution C:** 12 µg/mL of USP Cyclophosphamide Related Compound C RS in *Diluent*

**Standard solution D:** 15 µg/mL of USP Cyclophosphamide Related Compound D RS in *Diluent*.

[NOTE—Cyclophosphamide related compound D is free base ( $M_r$  = 260.66) and USP Cyclophosphamide Related Compound D RS is available as dihydrochloride salt ( $M_r$  = 333.58).]

**Standard solution E:** 12 µg/mL of USP Cyclophosphamide RS in *Diluent*

**Sample solution:** 20 mg/mL of Cyclophosphamide in *Diluent*

##### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture containing a fluorescent indicator

**Application volume:** 20 µL

**Developing solvent system:** Methylene chloride, glacial acetic acid, methanol, and water (50:25:15:12)

**Reagent A:** 3.16 g/L solution of potassium permanganate in water and 10% hydrochloric acid (1:1).

[NOTE—Mix in a small beaker at the time of use under a fume hood to generate chlorine gas, and immediately place the beaker with solution into closed TLC chamber (placed in a fume hood).]

**Reagent B:** Dissolve 250 mg of tetramethylbenzidine in 50 mL of dehydrated alcohol, and dilute with cyclohexane to 200 mL.

##### Analysis

**Samples:** *Standard solution A*, *Standard solution B*, *Standard solution C*, *Standard solution D*, *Standard solution E*, and *Sample solution*

[NOTE—Apply *Standard solution E* after the plate development in the *Developing solvent system*. Proceed as directed in the *Analysis* below.]

Develop with *Developing solvent system* over a path of 10 cm followed by drying at room temperature for 15 min in a fume hood. Develop again in the fresh portion of the *Developing solvent system* over a path of 10 cm followed by drying at room temperature for 15 min in a fume hood. Apply *Standard solution E* at the starting point of the plate. Dry the plate in an oven at 50° under a vacuum for 20 min or using a TLC heating plate at 50° for 20 min in a fume hood. Allow the plate to stand at room temperature for 5 min. Place the plate in a closed chromatography tank (placed in a fume hood) containing *Reagent A*, and leave the plate in the tank for at least 15 min. Remove the plate and place it in a fume hood for 15 min to remove the excess chlorine. Stain the plate by dipping it into *Reagent B* or spraying it with *Reagent B*. Examine the plate by visual evaluation.



**Acceptance criteria**

The spot of cyclophosphamide related compound A from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound A from *Standard solution A* (0.06%).

The spot of cyclophosphamide related compound B from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound B from *Standard solution B* (0.06%).

The spot of cyclophosphamide related compound C from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound C from *Standard solution C* (0.06%).

The spot of cyclophosphamide related compound D from the *Sample solution* is not more intense than the spot of cyclophosphamide related compound D from *Standard solution D* (0.06%).

The spot of any individual unspecified impurity in the *Sample solution* is not more intense than the spot of cyclophosphamide from *Standard solution E* (0.06%).

**Individual impurities:** See *Impurity Table 1*.

**Impurity Table 1**

Name	Retardation Factor	Acceptance Criteria, NMT (%)
Cyclophosphamide related compound D <sup>a</sup>	0.15	0.06
Cyclophosphamide related compound C <sup>b</sup>	0.20	0.06
Cyclophosphamide related compound B <sup>c</sup>	0.43	0.06
Cyclophosphamide related compound A <sup>d</sup>	0.90	0.06
Any unspecified impurity	—	0.06

<sup>a</sup> 3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate.

<sup>b</sup> 3-Aminopropyl dihydrogen phosphate.

<sup>c</sup> 3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.

<sup>d</sup> Bis(2-chloroethyl)amine hydrochloride.

**SPECIFIC TESTS****• LIMIT OF CHLORIDE**

**Sample solution:** Dissolve 2.0 g of Cyclophosphamide in 30 mL of water, and add 80 mL of isopropyl alcohol and 5 mL of 10% nitric acid.

**Analysis:** Titrate potentiometrically with 0.01 N silver nitrate VS. Perform a blank determination, and make any necessary correction (see *Titrimetry* (541)). Each 1.0 mL of 0.01 N silver nitrate equals 0.355 mg of chloride ion.

Calculate the percentage of chloride in the portion of Cyclophosphamide taken:

$$\text{Result} = [(V - B) \times N \times F \times 100] / [TN \times W \times (100 - A) / 100]$$

V = sample titrant volume (mL)

B = blank titrant volume (mL)

N = titrant normality

F = equivalence factor, 0.355 mg of chloride ion/mL of TN

TN = theoretical normality, 0.01 N

W = sample weight (mg)

A = assay correction for water

**Acceptance criteria:** NMT 0.033%

**• LIMIT OF PHOSPHATE**

**Diluent:** 0.2 g/mL of hydrochloric acid in water

**Solution A:** Heat 20 g of tin with 85 mL of hydrochloric acid until no more hydrogen is released. Allow to cool. Transfer 1.0 mL of this solution into a 10-mL volumetric flask, and dilute with *Diluent* to volume.

**Standard stock solution:** 0.72 g/L of monobasic potassium phosphate. Transfer 1.0 mL of this solution into a 100-mL volumetric flask, and dilute with water to volume. Prepare immediately before use.

**Standard solution:** *Standard stock solution* and water (1:49). Prepare immediately before use. [NOTE—This solution contains 100 µg/L of PO<sub>4</sub>.]

**Sample solution:** Dissolve 100 mg of Cyclophosphamide in water, and dilute to 100 mL.

**Analysis:** To the *Sample solution* add 4 mL of sulfomolybdic acid TS. Shake and add 0.1 mL of *Solution A*. Prepare a standard in the same manner using the *Standard solution*. After 10 min, compare the colors using 20 mL of each solution in color comparison tubes in diffused daylight, viewing vertically against a white background.

**Acceptance criteria:** Any color from the *Sample solution* is not more intense than that from the *Standard solution* (NMT 0.01%).

- **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Cyclophosphamide is sterile, it contains NMT 0.0625 USP Endotoxin Unit/mg of cyclophosphamide.
- **STERILITY TESTS (71):** Where the label states that Cyclophosphamide is sterile, it meets the requirements.
- **PH (791):** 3.9–7.1, in a solution (1 in 100), determined 30 min after its preparation
- **WATER DETERMINATION, Method I (921):** 5.7%–6.8%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers at a temperature between 2° and 30°.

- **LABELING:** Where the label states that Cyclophosphamide is sterile, the tests for *Bacterial Endotoxins Test* (85) and *Sterility Tests* (71) should be performed.

**• USP REFERENCE STANDARDS (11)**

USP Cyclophosphamide RS

USP Cyclophosphamide Related Compound A RS  
Bis(2-chloroethyl)amine hydrochloride.

C<sub>4</sub>H<sub>9</sub>Cl<sub>2</sub>N · HCl 178.49

USP Cyclophosphamide Related Compound B RS

3-(2-Chloroethyl)-2-oxo-2-hydroxy-1,3,6,2-oxadiazaphosphonane.

C<sub>7</sub>H<sub>16</sub>ClN<sub>2</sub>O<sub>3</sub>P 242.64

USP Cyclophosphamide Related Compound C RS

3-Aminopropyl dihydrogen phosphate.

C<sub>3</sub>H<sub>10</sub>NO<sub>4</sub>P 155.09

USP Cyclophosphamide Related Compound D RS

3-[2-(2-Chloroethylamino)ethylamino]propyl dihydrogen phosphate dihydrochloride.

C<sub>7</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>4</sub>P · 2HCl 333.58

USP Endotoxin RS

USP Propanolamine RS

3-Aminopropan-1-ol.

C<sub>3</sub>H<sub>9</sub>NO 75.11

**Cyclophosphamide for Injection****DEFINITION**

Cyclophosphamide for Injection is a sterile mixture of Cyclophosphamide with or without a suitable diluent. It contains NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous cyclophosphamide (C<sub>7</sub>H<sub>15</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>P).

**IDENTIFICATION****• A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)**

**Standard solution:** 20 mg/mL of USP Cyclophosphamide RS in chloroform

**Sample solution:** 20 mg/mL of cyclophosphamide in chloroform



**Chromatographic system**Application volume: 5  $\mu$ L

Developing solvent system: Chloroform, methanol, and ammonium hydroxide (75:20:5)

**Analysis****Samples:** *Standard solution* and *Sample solution*

Place the plate in an iodine chamber, and visualize the spots.

**Acceptance criteria:** Meets the requirements

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, both relative to the internal standard, as obtained in the Assay.

**ASSAY**• **PROCEDURE****Mobile phase:** Acetonitrile and water (30:70)**Internal standard solution:** Dissolve 185 mg of ethylparaben in 250 mL of alcohol in a 1000-mL volumetric flask, and dilute with water to volume.**Standard solution:** 0.5 mg/mL of anhydrous cyclophosphamide prepared as follows. Transfer a quantity of USP Cyclophosphamide RS into a suitable volumetric flask, add water equivalent to 50% of the final volume, and shake to dissolve. Add *Internal standard solution* equivalent to 10% of the final volume, and dilute with water to volume.**Sample stock solution:** Nominally 1 mg/mL of anhydrous cyclophosphamide in water from Cyclophosphamide for Injection**Sample solution:** 0.5 mg/mL of anhydrous cyclophosphamide prepared as follows. Transfer 25 mL of the *Sample stock solution* and 5 mL of the *Internal standard solution* into a 50-mL volumetric flask, and dilute with water to volume.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 195 nm**Column:** 3.9-mm  $\times$  30-cm; packing L1**Flow rate:** 1.5 mL/min**Injection volume:** 25  $\mu$ L**System suitability****Sample:** *Standard solution*

[NOTE—The relative retention times for cyclophosphamide and ethylparaben are about 0.7 and 1.0, respectively.]

**Suitability requirements****Resolution:** NLT 2 between cyclophosphamide and ethylparaben**Relative standard deviation:** NMT 2% from six replicate injections**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of anhydrous cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ) in the portion of Cyclophosphamide for Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

- $R_U$  = peak response ratio of cyclophosphamide to that of ethylparaben in the *Sample solution*  
 $R_S$  = peak response ratio of cyclophosphamide to that of ethylparaben in the *Standard solution*  
 $C_S$  = concentration of USP Cyclophosphamide RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of anhydrous cyclophosphamide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

**SPECIFIC TESTS**• **pH** (791)**Sample solution:** Nominally 20 mg/mL of anhydrous cyclophosphamide, determined 30 min after preparation**Acceptance criteria:** 3.0–9.0, but the range does not exceed 3 pH units

- **BACTERIAL ENDOTOXINS TEST** (85): NMT 0.20 USP Endotoxin Unit/mg of cyclophosphamide

- **STERILITY TESTS** (71): Meets the requirements

- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.

- **OTHER REQUIREMENTS:** It meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*.

**ADDITIONAL REQUIREMENTS****Change to read:**

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017). Storage at a temperature not exceeding 25° is recommended. It will withstand brief exposure to temperatures up to 30° but is to be protected from temperatures above 30°.
- **USP REFERENCE STANDARDS** (11)  
USP Cyclophosphamide RS  
USP Endotoxin RS

**Cyclophosphamide Tablets****DEFINITION**Cyclophosphamide Tablets contain NLT 90.0% and NMT 110.0% of the labeled amount of anhydrous cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ).**IDENTIFICATION**• **A. INFRARED ABSORPTION****Sample:** Extract a portion of finely powdered Tablets, equivalent to 50 mg of cyclophosphamide, with 25 mL of chloroform. Filter about 2 mL of the chloroform solution, mix the filtrate with 500 mg of potassium bromide, evaporate the chloroform, carefully removing the last trace of solvent in a small vacuum flask, and use the residue to prepare a potassium bromide dispersion.**Acceptance criteria:** The IR absorption spectrum of the *Sample* exhibits maxima, between 6.5 and 14  $\mu$ m, only at the same wavelengths as that of a similar preparation of USP Cyclophosphamide RS.

- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE****Mobile phase:** Acetonitrile and water (30:70)**Internal standard solution:** Dissolve 185 mg of ethylparaben in 250 mL of alcohol in a 1000-mL volumetric flask, and dilute with water to volume.**Standard solution:** 0.5 mg/mL of anhydrous cyclophosphamide prepared as follows. Transfer a quantity of USP Cyclophosphamide RS into a suitable volumetric flask, add water equivalent to 50% of the final volume, and shake to dissolve. Add *Internal standard solution* equivalent to 10% of the final volume, and dilute with water to volume.**Sample stock solution:** 1 mg/mL of anhydrous cyclophosphamide prepared as follows. Transfer NLT 10 Tablets to a suitable volumetric flask. Fill about half full with water, shake for 30 min, and dilute with water to



volume. Pass through fast, fluted filter paper, and discard the first 40–50 mL of the filtrate.

**Sample solution:** 0.5 mg/mL of anhydrous cyclophosphamide prepared as follows. Transfer 25 mL of the *Sample stock solution* and 5 mL of the *Internal standard solution* into a 50-mL volumetric flask, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 195 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 25 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for cyclophosphamide and ethylparaben are 0.7 and 1.0, respectively.]

#### Suitability requirements

**Resolution:** NLT 2 between cyclophosphamide and ethylparaben

**Relative standard deviation:** NMT 2% from six replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anhydrous cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ) in the portion of Tablets taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of cyclophosphamide to that of ethylparaben in the *Sample solution*

$R_S$  = peak response ratio of cyclophosphamide to that of ethylparaben in the *Standard solution*

$C_S$  = concentration of USP cyclophosphamide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of anhydrous cyclophosphamide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

**Medium:** Water; 900 mL, deaerated

**Apparatus 1:** 100 rpm

**Time:** 45 min

**Mobile phase:** Acetonitrile and water (30:70)

**Standard solution:** USP Cyclophosphamide RS in water at a concentration similar to that of the *Sample solution*

**Sample solution:** Pass a portion of solution under test through a suitable filter of 0.8-µm pore size.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 195 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 50 µL

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of anhydrous cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response of cyclophosphamide from the *Sample solution*

$r_S$  = peak response of cyclophosphamide from the *Standard solution*

$C_S$  = concentration of USP Cyclophosphamide RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of anhydrous cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ) is dissolved.

#### • UNIFORMITY OF DOSAGE UNITS (905)

##### Procedure for content uniformity

**Perchloric acid solution:** 2.35% (v/v) perchloric acid in water

**4-(p-Nitrobenzyl)pyridine solution:** 7.5 mg/mL of

4-(p-nitrobenzyl)pyridine in ethylene glycol

**Sodium hydroxide solution:** 20 mg/mL of sodium hydroxide in diluted alcohol

**Standard solution:** 500 µg/mL of USP Cyclophosphamide RS in water

**Sample solution:** 500 µg/mL of anhydrous cyclophosphamide prepared as follows. Place 1 Tablet in a suitable volumetric flask, fill the flask about two-thirds full of water, shake until the Tablet is completely disintegrated, dilute with water to volume, and filter. Discard the first 10 mL of the filtrate.

#### Instrumental conditions

**Mode:** Vis

**Analytical wavelength:** 560 nm

**Cell:** 1 cm

**Blank:** Water

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Place 2.0 mL each of the *Sample solution* and *Standard solution* in separate 27-mm × 170-mm test tubes. Treat each tube as follows. Add 0.7 mL of *Perchloric acid solution*, mix, and heat at 95° for 10 min. Cool, add 1.0 mL of sodium acetate TS, mix, add 1.6 mL of 4-(p-Nitrobenzyl)pyridine solution, mix, and heat at 95° for 10 min. Cool, and add 8.0 mL of *Sodium hydroxide solution*. Within 4 min after preparation, determine the absorbances of the solutions.

Calculate the percentage of the labeled amount of anhydrous cyclophosphamide ( $C_7H_{15}Cl_2N_2O_2P$ ) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP cyclophosphamide RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of anhydrous cyclophosphamide in the *Sample solution* (mg/mL)

**Acceptance criteria:** Meet the requirements

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in tight containers.

Storage at a temperature not exceeding 25° is recommended. Tablets will withstand brief exposure to temperatures up to 30° but are to be protected from temperatures above 30°.

#### • USP REFERENCE STANDARDS (11)

USP Cyclophosphamide RS

## Cyclopropane



$C_3H_6$  42.08

Cyclopropane.

Cyclopropane [75-19-4].



» Cyclopropane contains not less than 99.0 percent, by volume, of  $C_3H_6$ .

**Caution**—Cyclopropane is highly flammable. Do not use where it may be ignited.

**Packaging and storage**—Preserve in cylinders. [NOTE—Maintain cylinders of Cyclopropane at  $25 \pm 2^\circ$  for not less than 6 hours prior to withdrawing specimens for the tests and assay, and correct the results to  $25^\circ$  and 760 mm of mercury.]

**Labeling**—The label bears a warning that cyclopropane is highly flammable and is not to be used where it may be ignited.

**Acidity or alkalinity**—Add 0.3 mL of methyl red TS and 0.3 mL of bromothymol blue TS to 400 mL of boiling water, and boil the solution for 5 minutes. Pour 100 mL of the boiling solution into each of three color-comparison tubes marked A, B, and C, respectively. To tube B add 0.20 mL of 0.012 N hydrochloric acid, and to tube C add 0.40 mL of 0.012 N hydrochloric acid. Insert the stopper in each of the tubes, and cool them to room temperature. Pass 2000 mL of Cyclopropane through the solution in tube B at a rate requiring about 30 minutes for the passage of the gas: the color of the solution in tube B is no deeper orange-red than that in tube C and no deeper yellow-green than that in tube A.

NOTE—The various detector tubes called for in the respective tests are listed under *Reagents* in the section *Reagents, Indicators, and Solutions*.

**Carbon dioxide**—Place the container so that when its valve is opened, the gaseous phase can be sampled. Connect one end of a carbon dioxide detector tube to the container valve, and the other end to a gas flow meter. Pass 1000 mL of the Cyclopropane through the tube at a suitable rate: the indicator change corresponds to not more than 0.03%.

**Halogens**—Provide a 500-mL flask with a tightly fitting two-hole stopper. Through one opening pass a delivery tube bent at right angles and extending just beyond the lower surface of the stopper. Through the other opening insert a capillary tube bent at right angles and having a bore of  $1 \pm 0.2$  mm, in the same manner. Place in a 50-mL cylinder, having an internal diameter of  $2 \pm 0.25$  cm, 40 mL of a solution containing 850 mg of sodium carbonate in 1000 mL of water. Provide the cylinder with a two-hole stopper, and through one opening pass a right-angle delivery tube, having a bore of  $3 \pm 0.5$  mm, to within 2 mm of the bottom of the cylinder. The end of the delivery tube that extends out of the cylinder is provided with an enlargement  $8 \pm 0.5$  cm long having an internal diameter of  $2 \pm 0.25$  cm. Through the other opening in the stopper pass another right-angle delivery tube, having it extend just below the surface of the stopper. Collect 500 mL of Cyclopropane in the flask. By means of hydrostatic pressure, applied through the delivery tube, force the gas through the capillary tube, the water used being previously saturated with Cyclopropane. Ignite the gas, place the enlarged end of the delivery tube, connected with the cylinder, around the flame, extending the flame one-third of the way into the enlargement. Apply suction to the shorter delivery tube connected with the cylinder, thus drawing the spent gases through the sodium carbonate solution, the period of ignition of the 500 mL of Cyclopropane requiring approximately 30 minutes. Make any necessary correction for the amount of halogen in the volume of air used for the ignition of the gas. Transfer the sodium carbonate solution to a 500-mL volumetric flask, and rinse the cylinder thoroughly, collecting the rinsings in the flask. Dilute the solution with water to volume, and mix. To a 50-mL aliquot add sufficient nitric acid to make it acid to litmus paper, and then add 1 mL of acid in excess. Prepare a blank containing 0.50 mL of 0.0012 N hydrochloric acid and 4 mL of the sodium carbonate solution in 46 mL of water, acidify to litmus with nitric

acid, then add 1 mL of acid in excess and 1 mL of silver nitrate TS to each solution: after 5 minutes any opalescence in the solution representing the Cyclopropane does not exceed that in the blank (0.02% as chloride).

**Propylene, allene, and other unsaturated hydrocarbons**—Place the container so that when its valve is opened, the gaseous phase can be sampled. Connect one end of an olefin detector tube to the container valve, and the other end to a gas flow meter. Pass the Cyclopropane through the detector tube at a suitable rate: the color of the indicating layer of the tube contents matches the color standard after the passage of not less than 400 mL of Cyclopropane (0.9% as propylene).

**Assay**—Place the container so that when its valve is opened, the gaseous phase can be sampled. Withdraw 100 mL of Cyclopropane, accurately measured in a gas buret previously filled with mercury and equipped with a leveling bulb at the lower end. Connect one arm of the buret stopcock to a pipet that previously has been filled with sulfuric acid. By appropriate manipulation of the stopcock and the leveling bulb, transfer the gas between the pipet and the buret, bringing about sufficient contact of the gas with the acid to reduce the volume of unabsorbed gas to a minimum as measured in the buret. Not more than 1.0 mL of gas remains.

## Cycloserine



$C_3H_6N_2O_2$  102.09

3-Isloxazolidinone, 4-amino-, (R)-.

(+)-4-Amino-3-isoxazolidinone [68-41-7].

» Cycloserine has a potency of not less than 900  $\mu$ g of  $C_3H_6N_2O_2$  per mg.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Cycloserine RS

**Identification**—Dissolve about 1 mg in 10 mL of 0.1 N sodium hydroxide. To 1 mL of the resulting solution add 3 mL of 1 N acetic acid and 1 mL of a mixture, prepared 1 hour before use, of equal parts of sodium nitroprusside solution (1 in 25) and 4 N sodium hydroxide: a blue color gradually develops.

**Condensation products**—Its absorptivity (see *Ultraviolet-Visible Spectroscopy* (857)) at 285 nm, determined in a 0.1 N sodium hydroxide solution containing 0.40 mg per mL is not more than 0.80.

**Specific rotation** (781S): between  $108^\circ$  and  $114^\circ$ .

Test solution: 50 mg per mL, in 2 N sodium hydroxide.

**Crystallinity** (69S): meets the requirements.

**pH** (791): between 5.5 and 6.5, in a solution (1 in 10).

**Loss on drying** (731)—Dry about 100 mg in a capillary-stoppered bottle in vacuum at  $60^\circ$  for 3 hours: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.5%, the charred residue being moistened with 2 mL of nitric acid and 5 drops of sulfuric acid.

**Assay**—

**pH 6.8 Phosphate buffer**—Prepare as directed in *Buffer Solutions* under *Solutions* in the section *Reagents, Indicators, and Solutions*.

**Mobile phase**—Dissolve 0.5 g of sodium 1-decanesulfonate in 800 mL of water, add 50 mL of acetonitrile



and 5 mL of glacial acetic acid, and mix. Adjust with 1 N sodium hydroxide to a pH of 4.4. Filter, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Quantitatively dissolve an accurately weighed quantity of USP Cycloserine RS in pH 6.8 Phosphate buffer to obtain a solution having a known concentration of about 0.4 mg per mL.

**Assay preparation**—Transfer about 20 mg of Cycloserine, accurately weighed, to a 50-mL volumetric flask, dissolve in and dilute with pH 6.8 Phosphate buffer to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 219-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at about 30°. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.8; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for cycloserine. Calculate the quantity, in μg, of C<sub>3</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub> in each mg of Cycloserine taken by the formula:

$$50,000(C/W)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cycloserine RS in the *Standard preparation*; W is the quantity, in mg, of Cycloserine taken to prepare the *Assay preparation*; and  $r_U$  and  $r_S$  are the peak responses for cycloserine obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cycloserine Capsules

» Cycloserine Capsules contain not less than 90.0 percent and not more than 120.0 percent of the labeled amount of cycloserine (C<sub>3</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub>).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—  
USP Cycloserine RS

**Identification**—Shake a quantity of the contents of Capsules, equivalent to about 10 mg of cycloserine, with 100 mL of 0.1 N sodium hydroxide, and filter: 1 mL of the filtrate so obtained responds to the *Identification* test under *Cycloserine*.

**Dissolution** (711)—

**Medium**: pH 6.8 Phosphate buffer (see *Buffer Solutions* under *Solutions* in the section *Reagents, Indicators, and Solutions*); 900 mL.

**Apparatus 1**: 100 rpm.

**Time**: 30 minutes.

Determine the amount of C<sub>3</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub> dissolved by employing the following method.

pH 6.8 Phosphate buffer, *Mobile phase*, and *Chromatographic system*—Proceed as directed in the *Assay*.

**Standard solution**—Quantitatively dissolve an accurately weighed quantity of USP Cycloserine RS in pH 6.8 Phosphate buffer to obtain a solution having a known concentration of about 0.25 mg per mL.

**Test solution**—Use a filtered portion of the solution under test.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard solution* and the *Test solution* into the chro-

matograph, record the chromatograms, and measure the peak responses for cycloserine. Calculate the quantity, in mg, of cycloserine (C<sub>3</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub>) dissolved by the formula:

$$900C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cycloserine RS in the *Standard solution*; and  $r_U$  and  $r_S$  are the peak responses for cycloserine obtained from the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 80% (Q) of the labeled amount of C<sub>3</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub> is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Loss on drying** (731)—Dry about 100 mg of the contents of Capsules in a capillary-stoppered bottle in vacuum at 60° for 3 hours: it loses not more than 1.0% of its weight.

**Assay**—

pH 6.8 Phosphate buffer, *Mobile phase*, *Standard preparation*, and *Chromatographic system*—Proceed as directed in the *Assay* under *Cycloserine*.

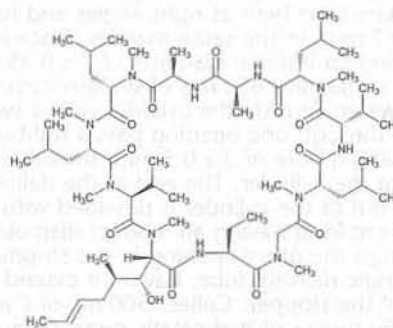
**Assay preparation**—Remove, as completely as possible, the contents of not fewer than 20 Capsules. Transfer an accurately weighed portion of the powder, equivalent to about 100 mg of cycloserine, to a 250-mL volumetric flask, dilute with pH 6.8 Phosphate buffer to volume, mix, and filter.

**Procedure**—Proceed as directed in the *Assay* under *Cycloserine*. Calculate the quantity, in mg, of cycloserine (C<sub>3</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub>) in the portion of Capsules taken by the formula:

$$250C(r_U / r_S)$$

in which the terms are as defined therein.

## Cyclosporine



C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub>

1202.61

Cyclo[(E)-(2S,3R,4R)-3-hydroxy-4-methyl-2-(methylamino)-6-octenyl]-L-2-aminobutyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl-L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl];  
[R-[R\*,R\*-(E)]-Cyclic(L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl-3-hydroxy-N,4-dimethyl-L-2-amino-6-octenyl-L-α-aminobutyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl)] [59865-13-3].

### DEFINITION

Cyclosporine contains NLT 97.0% and NMT 101.5% of cyclosporine A (C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub>), calculated on the dried basis.

### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.



**ASSAY****PROCEDURE**

**Mobile phase:** Acetonitrile, *tert*-butyl methyl ether, water, and phosphoric acid (430:50:520:1)

**Diluent:** Acetonitrile and water (1:1)

**System suitability solution:** 1.25 mg/mL of USP Cyclosporine Resolution Mixture RS in *Diluent*

**Standard solution:** 1.25 mg/mL of USP Cyclosporine RS in *Diluent*

**Sample solution:** 1.25 mg/mL of Cyclosporine in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4-mm × 25-cm; 3- to 5-μm packing L1; with 0.25-mm × 1-m stainless steel tubing connected to the column inlet

**Column temperature:** 80°. The tubing and column are maintained at 80°, to ensure that the *Mobile phase* entering the column is heated to 80°.

**Flow rate:** 1.2 mL/min

**Injection volume:** 20 μL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cyclosporine U and cyclosporine are 0.95 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.0 between cyclosporine U and cyclosporine, *System suitability solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cyclosporine

(C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub>) in the portion of Cyclosporine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak area of cyclosporine from the *Sample solution*

$r_S$  = peak area of cyclosporine from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

$P$  = potency of cyclosporine in USP Cyclosporine RS (mg/mg)

**Acceptance criteria:** 97.0%–101.5% on the dried basis

**IMPURITIES****Delete the following:**

• **HEAVY METALS, Method II (231):** NMT 20 ppm • (Official 1, Jan-2018)

**ORGANIC IMPURITIES**

**Mobile phase, Diluent, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.01 mg/mL of USP Cyclosporine RS in *Diluent*

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for cyclosporine U and cyclosporine are 0.95 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.0 between cyclosporine U and cyclosporine, *System suitability solution*

**Relative standard deviation:** NMT 10.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cyclosporine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times 100$$

$r_U$  = peak area of an individual impurity from the *Sample solution*

$r_S$  = peak area of cyclosporine from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

$P$  = potency of cyclosporine in USP Cyclosporine RS (mg/mg)

**Acceptance criteria**

Reporting threshold is 0.05%.

**Any individual impurity:** NMT 0.7%

**Total impurities:** NMT 1.5%

**SPECIFIC TESTS****Loss on Drying (731)**

**Sample:** 100 mg

**Analysis:** Dry in a capillary-stoppered bottle under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

**Acceptance criteria:** NMT 2.0%

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.

**USP REFERENCE STANDARDS (11)**

USP Cyclosporine RS

USP Cyclosporine Resolution Mixture RS

This is a 100:1 (w/w) mixture of cyclosporine and cyclosporine U. The chemical name for cyclosporine U is given below.

Cyclo[[*(E)*-(2*S*,3*R*,4*R*)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-aminobutyl-L-methylglycyl-L-methyl-L-leucyl-L-valyl-L-leucyl-L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl].  
C<sub>61</sub>H<sub>109</sub>N<sub>11</sub>O<sub>12</sub> 1188.58

**Cyclosporine Capsules**

» Cyclosporine Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cyclosporine (C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub>).

**Packaging and storage**—Preserve in tight containers, and store at controlled room temperature.

**Identification**—The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Dissolution (711)**—

WHERE CAPSULES CONTAIN LIQUID—

**Medium:** water; 500 mL.

**Apparatus 2:** 50 rpm.

**Time:** 15 minutes.

**Procedure**—Place 1 Capsule in each vessel, and allow the Capsule to sink to the bottom of the vessel before starting rotation of the blade. Observe the Capsules, and record the time taken for each Capsule shell to rupture.

**Tolerances**—The requirements are met if all of the Capsules tested rupture in not more than 15 minutes. If 1 or 2 of the Capsules rupture in more than 15 but not more than



30 minutes, repeat the test on 12 additional Capsules. Not more than 2 of the total of 18 Capsules tested rupture in more than 15 but not more than 30 minutes.

WHERE CAPSULES CONTAIN POWDER—

**Medium:** 0.1 N hydrochloric acid containing 0.5% of sodium lauryl sulfate; 1000 mL.

**Apparatus 1:** 150 rpm.

**Time:** 90 minutes.

Determine the amount of  $C_{62}H_{111}N_{11}O_{12}$  dissolved by employing the following method.

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, water, methanol, and phosphoric acid (900:450:50:0.5). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**Standard solution**—Quantitatively dissolve an accurately weighed quantity of USP Cyclosporine RS in *Dissolution Medium* to obtain a solution having a known concentration of about 0.001L mg per mL, L being the labeled quantity, in mg, of cyclosporine in each Capsule. Transfer 25.0 mL of this solution to a 50-mL volumetric flask, dilute with acetonitrile to volume, and mix. This solution contains about 0.0005L mg of USP Cyclosporine RS per mL.

**Test solution**—Filter a portion of the solution under test. Transfer 5.0 mL of the filtrate to a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L1 and is maintained at a constant temperature of about 80°. The flow rate is about 2 mL per minute. Chromatograph the *Standard solution*, and record the peak areas as directed for *Procedure*: the column efficiency is not less than 700 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the solution estimated to contain 0.1 mg of cyclosporine per mL, or 40  $\mu$ L of the solution estimated to contain 0.025 mg of cyclosporine per mL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of  $C_{62}H_{111}N_{11}O_{12}$  dissolved by the formula:

$$2000C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard solution*; and  $r_U$  and  $r_S$  are the cyclosporine peak areas obtained from the *Test solution* and the *Standard solution*, respectively.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{62}H_{111}N_{11}O_{12}$  is dissolved in 90 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Water Determination, Method I** (921)—For Capsules that contain powder, not more than 3.5% is found, using finely ground Capsule contents.

**Assay**—

WHERE CAPSULES CONTAIN LIQUID—

**Mobile phase and Chromatographic system**—Proceed as directed in the *Assay under Cyclosporine Injection*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyclosporine RS in dehydrated alcohol to obtain a solution having a known concentration of about 1 mg per mL. Use this solution promptly after preparation.

**Assay preparation**—Using a sharp blade, carefully cut open not fewer than 20 Capsules, and with the aid of dehydrated alcohol transfer the contents of the Capsules to a suitable volumetric flask. Wash the blade with dehydrated alcohol, and transfer the washings to the volumetric flask.

Dilute the contents of the volumetric flask with dehydrated alcohol to volume, and mix. Quantitatively dilute an accurately measured volume of this solution with dehydrated alcohol to obtain a solution having a concentration of about 1 mg of cyclosporine per mL.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of cyclosporine ( $C_{62}H_{111}N_{11}O_{12}$ ) in each Capsule taken by the formula:

$$(L/D)(CP/1000)(r_U / r_S)$$

in which L is the labeled quantity, in mg, of cyclosporine in each Capsule taken; D is the concentration, in mg per mL, of the *Assay preparation*, based on the labeled quantity of cyclosporine in the Capsules taken and the extent of dilution; C is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard preparation*; P is the purity, in  $\mu$ g per mg, of USP Cyclosporine RS; and  $r_U$  and  $r_S$  are the peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.

WHERE CAPSULES CONTAIN POWDER—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, water, methanol, and phosphoric acid (605:400:50:0.5). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**Diluting solvent**—Prepare a mixture of acetonitrile, tetrahydrofuran, and dehydrated alcohol (9:5:4).

**Standard preparation**—Transfer about 25 mg of USP Cyclosporine RS, accurately weighed, to a 25-mL volumetric flask. Add 2.5 mL of water, and sonicate for 10 minutes. Add about 10 mL of *Diluting solvent*, sonicate for 5 minutes, dilute with *Diluting solvent* to volume, and mix.

**Assay stock preparation**—Transfer the contents of 20 Capsules to a volumetric flask of such capacity, V, in mL, to make a final concentration of 10 mg of cyclosporine per mL. Add 0.1V mL of water to the flask, and sonicate for 10 minutes. Add 0.4V mL of *Diluting solvent* to the flask, and sonicate for 5 minutes. Dilute with *Diluting solvent* to volume, and mix.

**Assay preparation**—Transfer 5.0 mL of *Assay stock preparation* to a 50-mL volumetric flask, add 5 mL of water, dilute with *Diluting solvent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L13 and is maintained at a constant temperature of about 70°. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak areas as directed for *Procedure*: the column efficiency is not less than 700 theoretical plates; the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the quantity, in mg, of cyclosporine ( $C_{62}H_{111}N_{11}O_{12}$ ) in each Capsule taken by the formula:

$$10CV(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Cyclosporine RS in the *Standard preparation*; V is the volume, in mL, of the volumetric flask used to prepare the *Assay stock preparation*; and  $r_U$  and  $r_S$  are the cyclosporine peak areas obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Cyclosporine Injection

### DEFINITION

Cyclosporine Injection is a sterile solution of Cyclosporine in a suitable vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cyclosporine ( $C_{62}H_{111}N_{11}O_{12}$ ).

### IDENTIFICATION

#### A. THIN-LAYER CHROMATOGRAPHY

**Solution A:** 17 mg/mL of bismuth subnitrate in 20% acetic acid

**Solution B:** 400 mg/mL of potassium iodide

**Standard solution:** 0.5 mg/mL of USP Cyclosporine RS in methanol

**Sample solution:** Nominally 0.5 mg/mL of cyclosporine from Injection in methanol

#### Chromatographic system

(See Chromatography <621>, Thin-Layer Chromatography.)

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 10  $\mu$ L

**Developing solvent system 1:** Ethyl ether

**Developing solvent system 2:** Ethyl acetate, methyl ethyl ketone, water, and formic acid (60:40:2:1)

**Spray reagent 1:** Mix 5 mL of *Solution A* with 5 mL of *Solution B* and 20 mL of glacial acetic acid, and dilute with water to 100 mL. Prepare freshly.

**Spray reagent 2:** Hydrogen peroxide TS

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* to the plate. Allow the spots to dry in a current of air, place the plate in a suitable chromatographic chamber, and develop the chromatogram, using *Developing solvent system 1*, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow it to dry. Place the plate in a second chromatographic chamber, and develop the chromatogram in *Developing solvent system 2* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow it to dry. Spray the plate with *Spray reagent 1*. Immediately again spray the plate with *Spray reagent 2*. Cyclosporine appears as a brown spot having an  $R_f$  value of about 0.45.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. Disregard any spots at the origin.

- B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** Acetonitrile, methanol, water, and phosphoric acid (550:50:400:0.5)

**Standard solution:** 0.5 mg/mL of USP Cyclosporine RS in methanol. Use this solution promptly after preparation.

**Sample solution 1** (where it is represented as being in a single-dose container): Nominally 0.5 mg/mL of cyclosporine from Injection in methanol, prepared as follows. Using a suitable hypodermic needle and syringe, withdraw all of the withdrawable contents from 1 container of Injection, and dilute with methanol. Use this solution promptly after preparation.

**Sample solution 2** (where the label states the quantity of cyclosporine in a given volume): Nominally 0.5 mg/mL of cyclosporine from Injection in methanol, prepared as follows. Dilute a suitable aliquot of Injection

with methanol. Use this solution promptly after preparation.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** LC

**Detector:** UV 210 nm

**Column:** 4.6-mm  $\times$  25-cm; packing L16

**Column temperature:** 70°

**Flow rate:** 1 mL/min

**Injection volume:** 20  $\mu$ L

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

**Capacity factor:** NLT 3–NMT 10

**Column efficiency:** NLT 700 theoretical plates

**Tailing factor:** NMT 1.5

**Relative standard deviation:** NMT 1.5%

#### Analysis

**Samples:** *Standard solution* and *Sample solution 1* or *Sample solution 2*

Calculate the percentage of the labeled amount of cyclosporine ( $C_{62}H_{111}N_{11}O_{12}$ ) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from *Sample solution 1* or *Sample solution 2*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of *Sample solution 1* or *Sample solution 2* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### OTHER COMPONENTS

#### CONTENT OF ALCOHOL (where present)

**Internal standard solution:** *n*-Propyl alcohol and butyl alcohol (3:50)

**Standard stock solution:** 64 mg/mL of dehydrated alcohol in butyl alcohol

**Standard solution:** 12.8 mg/mL of alcohol, prepared as follows. Transfer a suitable aliquot of *Standard stock solution* to a suitable volumetric flask. Add *Internal standard solution*, using 24% of the final volume, and dilute with butyl alcohol to volume.

**Sample solution:** Nominally 12.8 mg/mL of alcohol from Injection, prepared as follows. Transfer a suitable aliquot of Injection to a suitable volumetric flask. Add *Internal standard solution*, using 24% of the final volume, and dilute with butyl alcohol to volume.

#### Chromatographic system

(See Chromatography <621>, System Suitability.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 2-mm  $\times$  2-m glass; packed with support S3

#### Temperatures

**Injection port:** 280°

**Detector:** 290°

**Column:** See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
145	0	145	8
145	32	270	0

**Carrier gas:** Nitrogen

**Flow rate:** 35 mL/min

**Injection volume:** 1  $\mu$ L. [NOTE—Make adjustments, if necessary, to obtain satisfactory chromatography.]



**System suitability**Sample: *Standard solution*Suitability requirements: [NOTE—The elution order is alcohol, *n*-propyl alcohol, and butyl alcohol.]

Relative standard deviation: NMT 2.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of alcohol (C<sub>2</sub>H<sub>5</sub>OH) in the portion of Injection taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak area ratio of alcohol to *n*-propyl alcohol from the *Sample solution*

$R_S$  = peak area ratio of alcohol to *n*-propyl alcohol from the *Standard solution*

$C_S$  = concentration of alcohol in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 80.0%–120.0% of the labeled amount

**SPECIFIC TESTS**• **BACTERIAL ENDOTOXINS TEST** (85)

Sample solution: Make a 1:10 dilution of the Injection with Water for Injection.

Analysis: Add 0.1 mL of *Sample solution* and 0.1 mL of appropriately constituted LAL reagent to a suitable pyrogen-free test tube. Mix on a vortex mixer for about 5 s.

Acceptance criteria: NMT 0.84 USP Endotoxin Unit/mg of cyclosporine

• **STERILITY TESTS** (71): Meets the requirements**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers.• **LABELING:** Label it to indicate that it is to be diluted with a suitable parenteral vehicle before intravenous infusion.• **USP REFERENCE STANDARDS** (11)

USP Cyclosporine RS

USP Endotoxin RS

**Cyclosporine Oral Solution****DEFINITION**Cyclosporine Oral Solution is a solution of Cyclosporine in a suitable vehicle. It contains NLT 90.0% and NMT 110.0% of the labeled amount of cyclosporine (C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub>).**IDENTIFICATION**• **A. THIN-LAYER CHROMATOGRAPHY**

Solution A: 17 mg/mL of bismuth subnitrate in 20% acetic acid

Solution B: 400 mg/mL of potassium iodide

Diluent: Methanol and chloroform (4:1)

Standard solution: 1 mg/mL of USP Cyclosporine RS in Diluent

Sample solution: Nominally 1 mg/mL of cyclosporine from Oral Solution in Diluent

Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

Adsorbent: 0.25-mm layer of chromatographic silica gel mixture

Application volume: 10 µL

Developing solvent system 1: Ethyl ether

Developing solvent system 2: Ethyl acetate, methyl ethyl ketone, water, and formic acid (60:40:2:1)

Spray reagent 1: Mix 5 mL of Solution A with 5 mL of Solution B and 20 mL of glacial acetic acid, and dilute with water to 100 mL. Prepare freshly.

Spray reagent 2: Hydrogen peroxide TS

**Analysis**Samples: *Standard solution* and *Sample solution*

Apply the *Standard solution* and the *Sample solution* to the plate. Allow the spots to dry in a current of air, place the plate in a suitable chromatographic chamber, and develop the chromatogram, using *Developing solvent system 1*, until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, mark the solvent front, and allow it to dry. Place the plate in a second chromatographic chamber, and develop the chromatogram in *Developing solvent system 2* until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chamber, and allow it to dry. Spray the plate with *Spray reagent 1*. Immediately again spray the plate with *Spray reagent 2*. Cyclosporine appears as a brown spot having an  $R_f$  value of about 0.45.

Acceptance criteria: The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*. Disregard any spots at the origin.• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.**ASSAY**• **PROCEDURE**

Mobile phase: Acetonitrile, methanol, water, and phosphoric acid (550: 50: 400: 0.5)

Diluent: Methanol and chloroform (4:1)

Standard solution: 1 mg/mL of USP Cyclosporine RS in Diluent. Use this solution promptly after preparation.

Sample solution: Nominally 1 mg/mL of cyclosporine from Oral Solution in Diluent. Use this solution promptly after preparation.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; packing L16

Column temperature: 50°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Capacity factor: 3–10

Column efficiency: NLT 700 theoretical plates

Tailing factor: NMT 1.5

Relative standard deviation: NMT 1.5%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of cyclosporine (C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub>) in the portion of Oral Solution taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

**OTHER COMPONENTS**• **CONTENT OF ALCOHOL** (where present)Internal standard solution: *n*-Propyl alcohol and butyl alcohol (3:50)

Standard stock solution: 50 mg/mL of dehydrated alcohol in butyl alcohol

Standard solution: 10 mg/mL of alcohol, prepared as follows. Transfer a suitable aliquot of *Standard stock so-*



lution to a suitable volumetric flask. Add *Internal standard solution*, using 24% of the final volume, and dilute with butyl alcohol to volume.

**Sample solution:** Nominally 10 mg/mL of alcohol from Oral Solution, prepared as follows. Transfer a suitable aliquot of Oral Solution to a suitable volumetric flask. Add *Internal standard solution*, using 24% of the final volume, and dilute with butyl alcohol to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 2-mm × 2-m glass; packed with support S3

**Temperatures**

**Injection port:** 280°

**Detector:** 290°

**Column:** See Table 1.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
145	0	145	8
145	32	270	0

**Carrier gas:** Nitrogen

**Flow rate:** 35 mL/min

**Injection volume:** 1 µL. [NOTE—Make adjustments if necessary to obtain satisfactory chromatography.]

#### System suitability

**Sample:** *Standard solution*. [NOTE—The elution order is alcohol, *n*-propyl alcohol, and butyl alcohol.]

#### Suitability requirements

**Relative standard deviation:** NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of alcohol (C<sub>2</sub>H<sub>5</sub>OH) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak area ratio of alcohol to *n*-propyl alcohol from the *Sample solution*

$R_S$  = peak area ratio of alcohol to *n*-propyl alcohol from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 80.0%–120.0% of the labeled amount

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements for oral solution packaged in single-unit containers
- **DELIVERABLE VOLUME** (698): Meets the requirements for oral solution packaged in multiple-unit containers

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers.
- **USP REFERENCE STANDARDS** (11)  
USP Cyclosporine RS

## Cyclosporine Compounded Ophthalmic Solution, Veterinary

#### DEFINITION

Cyclosporine Compounded Ophthalmic Solution, Veterinary contains NLT 90.0% and NMT 110.0% of the labeled amount of cyclosporine (C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub>).

Prepare Cyclosporine Compounded Ophthalmic Solution, Veterinary 10 mg/mL as follows (see *Pharmaceutical Compounding—Sterile Preparations* (797)).

Cyclosporine Oral Solution <sup>a</sup> equivalent to	100 mg of cyclosporine
Corn Oil, NF, a sufficient quantity to make	10 mL

<sup>a</sup> Sandimmune Oral Solution 100 mg/mL, Novartis Pharmaceuticals Corporation, East Hanover, NJ.

Mix the *Cyclosporine Oral Solution* with sufficient *Corn Oil* to bring to final volume and mix thoroughly. Pass the solution through a compatible sterile membrane filter of 0.22-µm pore size into a sterile ophthalmic container. Replace the tip and cap, and mix well. [NOTE—Cyclosporine Oral Solution Modified is not interchangeable and should not be used.]

#### ASSAY

##### PROCEDURE

**Mobile phase:** See Table 1.

Table 1

Time (min)	Acetonitrile (%)	Water (%)
0	60	40
40	90	10
45	90	10
45.05	60	40
55	60	40

**Standard solution:** 0.5 mg/mL of cyclosporine prepared from USP Cyclosporine RS in acetonitrile. Mix well to dissolve.

**Sample solution:** Transfer 0.5 mL of Ophthalmic Solution, Veterinary into a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix well. Allow the oil to separate from the solution. Once the top layer appears clear, transfer about 1 mL of the top layer into an amber HPLC vial.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV-Vis 208 nm

**Column:** 4.6-mm × 10-cm; 2.6-µm packing L1

**Column temperature:** 60°

**Flow rate:** 0.5 mL/min

**Injection volume:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for cyclosporine is about 34.0 min.]

#### Suitability requirements

**Tailing factor:** NMT 2.0

**Relative standard deviation:** NMT 2.0% for replicate injections

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of cyclosporine (C<sub>62</sub>H<sub>111</sub>N<sub>11</sub>O<sub>12</sub>) in the portion of Ophthalmic Solution, Veterinary taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of cyclosporine from the *Sample solution*

$r_S$  = peak response of cyclosporine from the *Standard solution*

$C_S$  = concentration of USP Cyclosporine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of cyclosporine in the *Sample solution* (mg/mL)



Acceptance criteria: 90.0%–110.0%

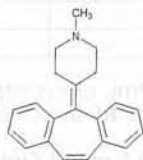
### SPECIFIC TESTS

- **STERILITY TESTS** (71): It meets the requirements.
- **PARTICULATE MATTER IN OPHTHALMIC SOLUTIONS** (789): It meets the requirements.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Package in sterile ophthalmic dropper bottles, protected from light. Store at controlled room temperature.
- **BEYOND-USE DATE:** In the absence of performing and completing a sterility test, the storage conditions for High-Risk Level CSPs in *Pharmaceutical Compounding—Sterile Preparations* (797) apply. After successful completion of sterility testing, NMT 180 days after the date on which it was compounded when stored at controlled room temperature.
- **LABELING:** Label it to indicate that it is for veterinary use only. State that it is intended for use in the eye and to not use if a precipitate is present. State the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** (11)  
USP Cyclosporine RS

## Cyproheptadine Hydrochloride



• HCl • 1½ H<sub>2</sub>O

$C_{21}H_{21}N \cdot HCl \cdot 1\frac{1}{2}H_2O$  350.88  
 $C_{21}H_{21}N \cdot HCl$  323.87  
 Piperidine, 4-(5H-dibenzo[a,d]cyclohepten-5-ylidene)-1-methyl-, hydrochloride, sesquihydrate;  
 4-(5H-Dibenzo[a,d]cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride sesquihydrate [41354-29-4].  
 Anhydrous [969-33-5].

### DEFINITION

Cyproheptadine Hydrochloride contains NLT 98.5% and NMT 100.5% of cyproheptadine hydrochloride ( $C_{21}H_{21}N \cdot HCl$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the cyproheptadine peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the test for *Organic Impurities*.
- **C.**  
*Sample solution:* 10 mg/mL of Cyproheptadine Hydrochloride in methanol  
*Analysis:* Place 1 drop of the *Sample solution* on a filter paper, dry, and view under short-wavelength UV light.  
*Acceptance criteria:* A bright blue fluorescence is observed.

### ASSAY

- **PROCEDURE**  
*Sample:* 250 mg of Cyproheptadine Hydrochloride  
*Diluent:* Mixture of alcohol and 0.01 N hydrochloric acid (50:5)  
*Blank:* 55 mL of *Diluent*  
*Titrimetric system*  
 (See *Titrimetry* (541).)

**Mode:** Direct titration

**Titrant:** 0.1 N sodium hydroxide VS

**Endpoint detection:** Potentiometric

**Analysis:** Dissolve the *Sample* in 55 mL of *Diluent*, and titrate with *Titrant*. Carry out a blank titration, and determine the volume added between the two points of inflection. Each mL of 0.1 N sodium hydroxide is equivalent to 32.39 mg of cyproheptadine hydrochloride ( $C_{21}H_{21}N \cdot HCl$ ).

**Acceptance criteria:** 98.5%–100.5% on the anhydrous basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%

### Delete the following:

- **HEAVY METALS, Method II** (231): NMT 30 ppm (Official 1, Jan-2018)

### ORGANIC IMPURITIES

**Buffer:** 6.1 g/L of monobasic potassium phosphate, prepared as follows. Dissolve 6.1 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid to a pH of 4.5, and dilute with water to 1000 mL.

**Solution A:** Acetonitrile and *Buffer* (40:60)

**Solution B:** Acetonitrile and *Buffer* (60:40)

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10.0	100	0
10.1	0	100
35.0	0	100

**System suitability solution:** 0.02 mg/mL each of USP Cyproheptadine Hydrochloride RS, USP Cyproheptadine Related Compound A RS, USP Amitriptyline Related Compound A RS, and USP Cyproheptadine Related Compound C RS in *Solution A*

**Standard solution:** 0.002 mg/mL each of USP Cyproheptadine Hydrochloride RS, USP Cyproheptadine Related Compound A RS, USP Amitriptyline Related Compound A RS, and USP Cyproheptadine Related Compound C RS in *Solution A* from the *System suitability solution*

**Sample solution:** 2.0 mg/mL of Cyproheptadine Hydrochloride in *Solution A*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 230 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 10 μL

### System suitability

[NOTE—See *Table 2* for the relative retention times.]

**Samples:** *System suitability solution* and *Standard solution*

### Suitability requirements

**Resolution:** NLT 7.0 between cyproheptadine and cyproheptadine related compound C, *System suitability solution*

**Relative standard deviation:** NMT 15.0% for cyproheptadine, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of cyproheptadine related compound A, amitriptyline related compound A, and



cyproheptadine related compound C in the portion of Cyproheptadine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of each related compound from the *Sample solution*  
 $r_s$  = peak response of the appropriate standard from the *Standard solution*  
 $C_s$  = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)  
 $C_u$  = concentration of Cyproheptadine Hydrochloride in the *Sample solution* (mg/mL)

Calculate the percentage of any individual unknown other impurity in the portion of Cyproheptadine Hydrochloride taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

- $r_u$  = peak response of any individual unknown impurity from the *Sample solution*  
 $r_s$  = peak response of cyproheptadine hydrochloride from the *Standard solution*  
 $C_s$  = concentration of USP Cyproheptadine Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_u$  = concentration of Cyproheptadine Hydrochloride in the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 2. Disregard peaks that are less than 0.05% of the cyproheptadine peak.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cyproheptadine related compound C <sup>a</sup>	0.7	0.15
Cyproheptadine	1.0	—
Amitriptyline related compound A <sup>b</sup>	2.6	0.15
Cyproheptadine related compound A <sup>c</sup>	3.9	0.15
Any individual unknown impurity	—	0.10
Total impurities	—	0.5

<sup>a</sup> 5-(1-Methyl-piperidin-4-yl)-5H-dibenzo[a,d]cyclohepten-5-ol.

<sup>b</sup> Dibenzosuberone.

<sup>c</sup> 5H-Dibenzo[a,d]cycloheptene.

## SPECIFIC TESTS

### • ACIDITY

**Sample solution:** 40.0 mg/mL of Cyproheptadine Hydrochloride in methanol

**Analysis:** To 25 mL of the *Sample solution* add methyl red TS, and titrate with 0.10 N sodium hydroxide.

**Acceptance criteria:** NMT 0.15 mL of 0.10 N sodium hydroxide is required (0.05% as HCl).

### • WATER DETERMINATION, Method I (921): 7.0%–9.0%

## ADDITIONAL REQUIREMENTS

### • PACKAGING AND STORAGE: Preserve in well-closed containers.

### • USP REFERENCE STANDARDS (11)

USP Amitriptyline Related Compound A RS  
Dibenzosuberone.

C<sub>15</sub>H<sub>12</sub>O 208.26

USP Cyproheptadine Hydrochloride RS

USP Cyproheptadine Related Compound A RS  
5H-Dibenzo[a,d]cycloheptene.

C<sub>15</sub>H<sub>12</sub> 192.26

USP Cyproheptadine Related Compound C RS

5-(1-Methyl-piperidin-4-yl)-5H-dibenzo[a,d]cyclohepten-5-ol.

C<sub>21</sub>H<sub>23</sub>NO 305.41

## Cyproheptadine Hydrochloride Oral Solution

» Cyproheptadine Hydrochloride Oral Solution contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of cyproheptadine hydrochloride (C<sub>21</sub>H<sub>21</sub>N · HCl).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards (11)**—

USP Cyproheptadine Hydrochloride RS

**Identification**—Place about 50 mL of Oral Solution in a separator, add 25 mL of sodium bicarbonate solution (2 in 100), and extract with three 15-mL portions of isooctane. Wash the combined isooctane extracts with 15 mL of sodium bicarbonate solution (2 in 100), and discard the washing. Evaporate the isooctane solution on a steam bath to dryness, and dissolve the residue in 1 mL of carbon disulfide, filtering if necessary. Determine the IR absorption spectrum as directed under *Identification—Organic Nitrogenous Bases* (181), obtaining the spectrum of USP Cyproheptadine Hydrochloride RS as directed: the Oral Solution meets the requirements of the test.

**pH (791):** between 3.5 and 4.5.

**Assay**—

**Methanesulfonic acid solution, Mobile phase, and Chromatographic system**—Proceed as directed in the Assay under Cyproheptadine Hydrochloride Tablets.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyproheptadine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.02 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Oral Solution, equivalent to about 2 mg of cyproheptadine hydrochloride, to a 100-mL volumetric flask. Dilute with *Mobile phase* to volume, and mix. Pass the solution through a filter having a 0.45-μm or finer porosity.

**Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of cyproheptadine hydrochloride (C<sub>21</sub>H<sub>21</sub>N · HCl) in the portion of Oral Solution taken by the formula:

$$100C(r_u / r_s)$$

in which C is the concentration, in mg per mL, of USP Cyproheptadine Hydrochloride RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the cyproheptadine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cyproheptadine Hydrochloride Tablets

» Cyproheptadine Hydrochloride Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>21</sub>H<sub>21</sub>N · HCl.



**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Cyproheptadine Hydrochloride RS

**Identification**—Tablets meet the requirements under *Identification—Organic Nitrogenous Bases* (181).

**Dissolution** (711)—

*Medium*: 0.1 N hydrochloric acid; 900 mL.

*Apparatus 2*: 50 rpm.

*Time*: 30 minutes.

**Procedure**—Determine the amount of  $C_{21}H_{21}N \cdot HCl$  dissolved by employing UV absorption at the wavelength of maximum absorbance at about 285 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Cyproheptadine Hydrochloride RS in the same *Medium*.

**Tolerances**—Not less than 80% (Q) of the labeled amount of  $C_{21}H_{21}N \cdot HCl$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Assay**—

**Methanesulfonic acid solution**—Prepare a solution of methanesulfonic acid in water (3:1000).

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, isopropyl alcohol, and *Methanesulfonic acid solution* (20:15:65); while mixing adjust with triethylamine to a pH of  $4.0 \pm 0.05$ . Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyproheptadine Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 0.08 mg per mL.

**Assay preparation**—Transfer a number of Tablets, accurately weighed, equivalent to 80 mg of cyproheptadine hydrochloride, to a 1-liter volumetric flask, dissolve by sonication in 500 mL of *Mobile phase* for 15 minutes, and agitate for 30 minutes. Dilute with *Mobile phase* to volume, and mix. Pass through a filter having a  $0.45\text{-}\mu\text{m}$  or finer porosity.

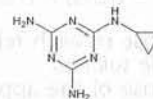
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 285-nm detector and a  $3.9\text{-mm} \times 15\text{-cm}$  column that contains packing L1. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 2.5; and the relative standard deviation for replicate injections is not more than 2%.

**Procedure**—Separately inject equal volumes (about 10  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{21}H_{21}N \cdot HCl$  in each of the Tablets taken by the formula:

$$1000(C/N)(r_U/r_S)$$

in which C is the concentration, in mg per mL, of USP Cyproheptadine Hydrochloride RS in the *Standard preparation*; N is the number of Tablets taken for the *Assay preparation*; and  $r_U$  and  $r_S$  are the cyproheptadine peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Cyromazine



$C_6H_{10}N_6$  166.18

N-Cyclopropyl-1,3,5-triazine-2,4,6-triamine.

2-Cyclopropylamino-4,6-diamino-s-triazine [66215-27-8].

» Cyromazine contains not less than 98.0 percent and not more than 102.0 percent of  $C_6H_{10}N_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Cyromazine RS

**Identification**—

A: *Infrared Absorption* (197K).

B: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Melting range** (741): between  $219^\circ$  and  $226^\circ$ .

**Loss on drying** (731)—Dry it at  $105^\circ$  to a constant weight: it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Assay**—

**Mobile phase**—Mix 930 mL of water, 3.72 g of dibasic potassium phosphate, and 6.48 g of monobasic potassium phosphate. Add 50 mL of methanol and 20 mL of acetonitrile, and mix. Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Cyromazine RS in methanol to obtain a solution having a known concentration of about 0.50 mg per mL. Dilute an aliquot of the resulting solution with *Mobile phase* to obtain a solution having a known concentration of about 10  $\mu\text{g}$  per mL.

**Assay preparation**—Dissolve an accurately weighed quantity of Cyromazine in methanol to obtain a solution having a known concentration of about 0.50 mg per mL. Dilute an aliquot of the resulting solution with *Mobile phase* to obtain a solution having a known concentration of about 10  $\mu\text{g}$  per mL.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 214-nm detector and a  $4.6\text{-mm} \times 25\text{-cm}$  column that contains  $5\text{-}\mu\text{m}$  packing L1. The flow rate is about 2.0 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 3000 theoretical plates; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the content, in percentage, of  $C_6H_{10}N_6$  in the portion of Cyromazine taken by the formula:

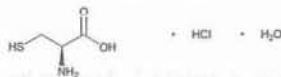
$$100(C_S/C_U)(r_U/r_S)$$

in which  $C_S$  is the concentration, in  $\mu\text{g}$  per mL, of USP Cyromazine RS in the *Standard preparation*;  $C_U$  is the concentration, in  $\mu\text{g}$  per mL, of Cyromazine in the *Assay preparation*;



and  $r_U$  and  $r_S$  are the peak responses obtained from the Assay preparation and the Standard preparation, respectively.

## Cysteine Hydrochloride



$C_3H_7NO_2S \cdot HCl \cdot H_2O$  175.63

$C_3H_7NO_2S \cdot HCl$  157.62

L-Cysteine hydrochloride monohydrate [7048-04-6].  
Anhydrous [52-89-1].

### DEFINITION

Cysteine Hydrochloride is L-cysteine hydrochloride monohydrate and contains NLT 98.5% and NMT 101.5% of L-cysteine hydrochloride ( $C_3H_7NO_2S \cdot HCl$ ), calculated on the dried basis.

### IDENTIFICATION

#### • A. INFRARED ABSORPTION (197K)

### ASSAY

#### • PROCEDURE

**Sample:** 250 mg of Cysteine Hydrochloride

**Blank:** Proceed as directed in the Analysis without the Sample.

**Titrimetric system**

(See *Titrimetry* (541).)

**Mode:** Residual titration

**Titrant:** 0.1 N iodine VS

**Back-titrant:** 0.1 N sodium thiosulfate VS

**Endpoint detection:** Visual

**Analysis:** Transfer the Sample to an iodine flask. Add 20.0 mL of water and 4 g of potassium iodide, and mix. Cool the solution in an ice bath, and add 5 mL of 3 N hydrochloric acid and 25.0 mL of 0.1 N iodine VS. Insert the stopper, and allow to stand in the dark for 20 min, while remaining in the ice bath. Titrate the excess iodine with the Back titrant. Add 3 mL of starch TS as the endpoint is approached. Perform the Blank determination.

Calculate the percentage of cysteine hydrochloride ( $C_3H_7NO_2S \cdot HCl$ ) in the portion of Cysteine Hydrochloride taken:

$$\text{Result} = \{[(V_B - V_S) \times N \times F]/W\} \times 100$$

$V_B$  = Back-titrant volume consumed by the Blank (mL)

$V_S$  = Back-titrant volume consumed by the Sample (mL)

$N$  = actual normality of the Back-titrant (mEq/mL)

$F$  = equivalency factor, 157.6 mg/mEq

$W$  = Sample weight (mg)

Acceptance criteria: 98.5%–101.5% on the dried basis

### IMPURITIES

#### • RESIDUE ON IGNITION (281): NMT 0.4%

#### • CHLORIDE AND SULFATE (221), Sulfate

**Standard solution:** 0.10 mL of 0.020 N sulfuric acid

**Sample:** 0.33 g of Cysteine Hydrochloride

Acceptance criteria: NMT 0.03%

#### • IRON (241): NMT 30 ppm

### Delete the following:

#### • HEAVY METALS (231), Method I: NMT 15 ppm (Official 1-Jan-2018)

### • RELATED COMPOUNDS

**N-Ethylmaleimide solution:** 40 mg/mL of N-ethylmaleimide in alcohol

**Standard stock solution:** Dissolve 20 mg of USP L-Cysteine Hydrochloride RS in 10.0 mL of water. Add 10.0 mL of N-Ethylmaleimide solution, and mix. Allow the solution to stand for 5 min before using.

**Standard solution:** 0.05 mg/mL from Standard stock solution in water. [NOTE—This solution has a concentration equivalent to 0.5% of that of the Sample solution.]

**System suitability solution:** Transfer 10 mg of USP L-Tyrosine RS and 10 mL of the Standard stock solution to a 25-mL volumetric flask. Dilute with water to volume.

**Sample stock solution:** Transfer 0.2 g of Cysteine Hydrochloride to a 10-mL volumetric flask. Dissolve and dilute with water to volume.

**Sample solution:** To 5.0 mL of the Sample stock solution add 5.0 mL of N-Ethylmaleimide solution, and mix. Allow the solution to stand for 5 min before using.

### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel mixture

**Application volume:** 5  $\mu$ L

**Developing solvent system:** Butyl alcohol, glacial acetic acid, and water (3:1:1)

**Spray reagent:** 2 mg/mL of ninhydrin in a mixture of butyl alcohol and 2 N acetic acid (95:5)

### System suitability

**Suitability requirements:** The chromatogram of the System suitability solution exhibits two clearly separated spots.

### Analysis

**Samples:** Standard solution, System suitability solution, and Sample solution

After air-drying the plate, spray with Spray reagent, and heat between 100° and 105° for 15 min. Examine the plate under white light.

**Acceptance criteria:** Any secondary spot of the Sample solution is not larger or more intense than the principal spot of the Standard solution.

**Individual impurities:** NMT 0.5%

**Total impurities:** NMT 2.0%

### SPECIFIC TESTS

#### • OPTICAL ROTATION (781S), Procedures, Specific Rotation

**Sample solution:** 80 mg/mL in 6 N hydrochloric acid

**Acceptance criteria:** +5.7° to +6.8°

### Change to read:

#### • LOSS ON DRYING (731)

**Analysis:** Dry at room temperature for 24 h in a vacuum desiccator using a suitable desiccant and maintaining a pressure of NMT 5 mm of mercury (Hg).▲USP40

**Acceptance criteria:** 8.0%–12.0%

### ADDITIONAL REQUIREMENTS

#### • PACKAGING AND STORAGE: Preserve in well-closed containers.

#### • USP REFERENCE STANDARDS (11)

USP L-Cysteine Hydrochloride RS

USP L-Tyrosine RS

## Cysteine Hydrochloride Injection

» Cysteine Hydrochloride Injection is a sterile solution of Cysteine Hydrochloride in Water for In-



jection. It contains not less than 85.0 percent and not more than 115.0 percent of the labeled amount of  $C_3H_7NO_2S \cdot HCl \cdot H_2O$ .

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP L-Cysteine Hydrochloride RS

USP Endotoxin RS

**Identification**—

**A:** To 2 mL of Injection add 3 mL of water, and mix. Add 10 mL of cupric sulfate TS: a bluish-gray precipitate is formed.

**B:** To 2 mL of Injection add 3 mL of water, and mix. Add 2 mL of 3 N sodium hydroxide and 2 drops of sodium nitro-ferricyanide solution (1 in 20): a red-purple color is produced, and it rapidly changes to yellow.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.7 USP Endotoxin Unit per mg of cysteine hydrochloride.

**pH** (791): between 1.0 and 2.5.

**Delete the following:**

• **Heavy metals, Method II** (231): 2 ppm. • (Official 1-Jan-2018)

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—

**Standard stock preparation**—Dissolve an accurately weighed quantity of USP L-Cysteine Hydrochloride RS in nitrogen-saturated water to obtain a solution having a known concentration of about 1 mg per mL.

**Standard preparation**—Transfer 20.0 mL of *Standard stock preparation* to a 200-mL volumetric flask, dilute with nitrogen-saturated 1.0 N sodium hydroxide to volume, and mix.

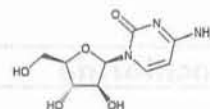
**Assay preparation**—Dilute an accurately measured volume of Injection, equivalent to about 250 mg of cysteine hydrochloride, quantitatively and stepwise with nitrogen-saturated 1.0 N sodium hydroxide, to obtain a solution having a concentration of about 0.1 mg per mL.

**Procedure**—Transfer a suitable amount of *Standard preparation* to a polarographic cell. With mercury dropping from the electrode, lower the dropping mercury electrode of a polarograph so that the end is submerged in the liquid. Bubble oxygen-free, water-saturated nitrogen through the liquid for 15 minutes. Record the polarogram from  $-0.2$  volt to  $-1.10$  volts, using a saturated calomel electrode as the reference electrode. In a similar manner, record the polarograms obtained using portions of the *Assay preparation* and of the nitrogen-saturated 1.0 N sodium hydroxide. Determine the height of the diffusion current wave at  $-0.4$  volt. Calculate the quantity, in mg, of  $C_3H_7NO_2S \cdot HCl \cdot H_2O$  in each mL of the Injection taken by the formula:

$$2500(C/V)[(i_a)_U / (i_a)_S]$$

in which C is the concentration, in mg per mL, of USP L-Cysteine Hydrochloride RS. In the *Standard preparation*, V is the volume, in mL, of Injection taken; and  $(i_a)_U$  and  $(i_a)_S$  are the observed diffusion currents, corrected for the diffusion current of the 0.1 N sodium hydroxide, of the *Assay preparation* and the *Standard preparation*, respectively.

## Cytarabine



$C_9H_{13}N_3O_5$

243.22

2(1H)-Pyrimidinone, 4-amino-1-β-D-arabinofuranosyl-;

1-β-D-Arabinofuranosylcytosine [147-94-4].

**DEFINITION**

Cytarabine contains NLT 98.0% and NMT 102.0% of cytarabine ( $C_9H_{13}N_3O_5$ ), calculated on the dried basis.

**IDENTIFICATION**

• **A. INFRARED ABSORPTION** (197M)

**Sample:** Previously dried at a pressure not exceeding 5 mm of mercury at 60° for 3 h

**Acceptance criteria:** Meets the requirements

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**

• **PROCEDURE**

**Solution A:** 0.73 g/L of monobasic sodium phosphate and 1.4 g/L of dibasic sodium phosphate in water

**Mobile phase:** Methanol and *Solution A* (5:95)

**Standard solution:** 0.1 mg/mL of USP Cytarabine RS in water

**System suitability solution:** 0.1 mg/mL each of USP Uracil Arabinoside RS and USP Cytarabine RS in water prepared as follows. Dissolve USP Uracil Arabinoside RS in *Standard solution*.

**Sample solution:** 0.1 mg/mL of Cytarabine in water

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 μL

[NOTE—After chromatography has been completed, flush the column with a mixture of water and methanol (7:3).]

**System suitability**

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for cytarabine and uracil arabinoside are about 1.0 and 1.3, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.5 between cytarabine and uracil arabinoside, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cytarabine ( $C_9H_{13}N_3O_5$ ) in the portion of Cytarabine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Cytarabine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cytarabine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis



**IMPURITIES**

- **RESIDUE ON IGNITION** (281): NMT 0.5%

Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1-Jan-2018)

- **ORGANIC IMPURITIES**

**Buffer:** 0.01 M monobasic sodium phosphate and 0.01 M dibasic sodium phosphate. Adjust with 0.1 M sodium hydroxide or 0.1 M phosphoric acid to a pH of 7.0.

**Solution A:** Methanol and *Buffer* (2:98). Prepare this solution fresh daily.

**Solution B:** Methanol and *Buffer* (30:70). Prepare this solution fresh daily.

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
20	0	100
25	0	100
30	100	0
50	100	0

**System suitability solution:** 0.02 mg/mL of uridine, 0.02 mg/mL of USP Uracil Arabinoside RS, and 5.0 mg/mL of USP Cytarabine RS in water

**Standard solution:** 4 µg/mL of USP Cytarabine RS in water

**Sample solution:** 5 mg/mL of Cytarabine in water. Prepare this solution fresh daily.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 1.25 between cytarabine and uridine, *System suitability solution*

**Relative standard deviation:** NMT 3.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Cytarabine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of USP Cytarabine RS from the *Standard solution*

$C_S$  = concentration of USP Cytarabine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Cytarabine in the *Sample solution* (mg/mL)

$F$  = relative response factor for each individual impurity, see *Table 2*

**Acceptance criteria**

**Individual impurities:** See *Table 2*.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT(%)
Impurity 1	0.38	1.5	0.10
Impurity 2	0.43	1.5	0.10
Uracil <sup>a</sup>	0.55	2.5	0.10
Uridine <sup>b</sup>	1.14	1.5	0.10
Uracil arabinoside <sup>c</sup>	1.62	1.34	0.30
Any individual unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.30

<sup>a</sup> Pyrimidine-2,4(1H,3H)-dione.

<sup>b</sup> 1-β-D-Ribofuranosyluracil.

<sup>c</sup> 2,4(1H,3H)-Pyrimidinedione, 1-β-D-arabinofuranosyl-.

**SPECIFIC TESTS**

- **OPTICAL ROTATION, Specific Rotation** (781S)

**Sample solution:** 10 mg/mL in water

**Acceptance criteria:** +154° to +160°

- **LOSS ON DRYING** (731)

**Analysis:** Dry under vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h.

**Acceptance criteria:** NMT 1.0%

- **STERILITY TESTS** (71): Where the label states that Cytarabine is sterile, it meets the requirements.

- **BACTERIAL ENDOTOXINS TEST** (85): Where the label states that Cytarabine is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.07 USP Endotoxin Unit/mg of cytarabine.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.

- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

- **USP REFERENCE STANDARDS** (11)

USP Cytarabine RS

USP Endotoxin RS

USP Uracil Arabinoside RS

2,4(1H,3H)-Pyrimidinedione, 1-β-D-arabinofuranosyl-.

C<sub>9</sub>H<sub>12</sub>N<sub>2</sub>O<sub>6</sub> 244.23

## Cytarabine for Injection

**DEFINITION**

Cytarabine for Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of cytarabine (C<sub>9</sub>H<sub>13</sub>N<sub>3</sub>O<sub>5</sub>).

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY**

- **PROCEDURE**

**Solution A:** 0.73 g/L of monobasic sodium phosphate and 1.4 g/L of dibasic sodium phosphate in water

**Mobile phase:** Methanol and *Solution A* (5:95)

**Standard solution:** 0.1 mg/mL of USP Cytarabine RS in water

**System suitability solution:** 0.1 mg/mL each of USP Uracil Arabinoside RS and USP Cytarabine RS in water prepared as follows. Dissolve USP Uracil Arabinoside RS in *Standard solution*.



**Sample solution:** 0.1 mg/mL of cytarabine in water prepared as follows. Separately constitute 5 vials of Cytarabine for Injection in a volume of water corresponding to the volume specified in the labeling. Pool and mix the constituted solutions in a suitable container. Transfer a volume of the constituted solution, nominally equivalent to 100 mg of cytarabine, into a 100-mL volumetric flask, and dilute with water to volume. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, and dilute with water to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L1

**Flow rate:** 1.0 mL/min

**Injection volume:** 10 µL

After chromatography has been completed, flush the column with a mixture of water and methanol (7:3).

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

[NOTE—The relative retention times for cytarabine and uracil arabinoside are about 1.0 and 1.3, respectively.]

#### Suitability requirements

**Resolution:** NLT 2.5 between cytarabine and uracil arabinoside, *System suitability solution*

**Relative standard deviation:** NMT 2.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of cytarabine ( $C_9H_{13}N_3O_5$ ) in the portion of Cytarabine for Injection taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

$r_u$  = peak response from the *Sample solution*

$r_s$  = peak response from the *Standard solution*

$C_s$  = concentration of USP Cytarabine RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of cytarabine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

#### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

#### SPECIFIC TESTS

- **PH** (791)

**Sample solution:** 10 mg/mL of cytarabine

**Acceptance criteria:** 4.0–6.0

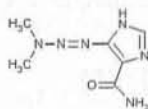
- **WATER DETERMINATION, Method I** (921): NMT 3.0%
- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.07 USP Endotoxin Unit/mg of cytarabine.
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.
- **STERILITY TESTS** (71): Meets the requirements
- **OTHER REQUIREMENTS:** The drug substance in the vial meets the requirements for *Cytarabine*.

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging*.
- **LABELING:** It meets the requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*.
- **USP REFERENCE STANDARDS** (11)
  - USP Cytarabine RS
  - USP Endotoxin RS
  - USP Uracil Arabinoside RS
  - 2,4(1H,3H)-Pyrimidinedione, 1-beta-D-arabinofuranosyl-,  $C_9H_{12}N_2O_6$  244.20



## Dacarbazine



$C_6H_{10}N_6O$  182.18  
1*H*-Imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazenyl)-  
5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide  
[4342-03-4].

» Dacarbazine contains not less than 97.0 percent and not more than 102.0 percent of  $C_6H_{10}N_6O$ .

**Caution**—Great care should be taken in handling Dacarbazine, as it is a potent cytotoxic agent.

**Packaging and storage**—Preserve in tight, light-resistant containers, in a refrigerator.

### USP Reference standards (11)—

USP Dacarbazine RS  
USP Dacarbazine Related Compound A RS  
5-Aminoimidazole-4-carboxamide hydrochloride.  
USP Dacarbazine Related Compound B RS  
2-Azahypoxanthine.  
 $C_4H_3N_5O \cdot H_2O$  155.12

**Identification**—The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Dacarbazine RS.

**Residue on ignition** (281): not more than 0.1%.

**Related compounds**—Dissolve an accurately weighed quantity of Dacarbazine in 0.1 N hydrochloric acid to obtain a solution having a concentration of 40 mg per mL, and apply 5  $\mu$ L of the solution to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Apply, separately, 5  $\mu$ L of a methanolic solution containing 0.40 mg of USP Dacarbazine Related Compound A RS per mL, and 5  $\mu$ L of an aqueous solution containing 0.40 mg of USP Dacarbazine Related Compound B RS per mL. Develop the chromatogram in a mixture of butanol, water, and acetic acid (5:2:1), until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by viewing under short-wavelength UV light: any spots obtained from the test solution are not greater in size or intensity than the spots, occurring at the respective  $R_f$  values, produced by the Standard solutions, corresponding to not more than 1.0% of dacarbazine related compound A and not more than 1.0% of dacarbazine related compound B.

**Assay**—[NOTE—Throughout this procedure, avoid exposing Dacarbazine and its solutions to light.]

**Standard preparations**—Transfer about 30 mg of USP Dacarbazine RS, accurately weighed, to a 50-mL volumetric flask, add 0.1 N hydrochloric acid to volume, and mix (Standard stock solution). Dilute a portion of Standard stock solution quantitatively and stepwise with 0.1 N hydrochloric acid to obtain an Acidic standard preparation having a known concentration of about 6  $\mu$ g per mL. Dilute a portion of Standard stock solution quantitatively and stepwise with pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) to obtain a Neutral standard preparation having a known concentration of about 6  $\mu$ g per mL.

**Assay preparations**—Prepare as directed under *Standard preparations*, except to use about 30 mg of Dacarbazine, accurately weighed.

**Procedure**—Concomitantly determine the absorbances of the Acidic standard preparation and the Acidic assay preparation in 1-cm cells at the wavelength of maximum absorbance at about 323 nm, with a suitable spectrophotometer, using 0.1 N hydrochloric acid as the blank. Concomitantly determine the absorbances of the Neutral standard preparation and the Neutral assay preparation in 1-cm cells at the wavelength of maximum absorbance at about 329 nm, using pH 7.0 phosphate buffer (see *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) as the blank. Calculate the quantity, in mg, of  $C_6H_{10}N_6O$  in the portion of Dacarbazine taken by the formula:

$$5C[(A_{323} + A_{329})_U / (A_{323} + A_{329})_S]$$

in which C is the concentration, in  $\mu$ g per mL, of USP Dacarbazine RS in the *Standard preparations*, and the parenthetical expressions are the sums of the absorbances of the *Assay preparations* (U) and the *Standard preparations* (S), respectively, measured at the wavelengths indicated by the subscripts.

## Dacarbazine for Injection

### DEFINITION

Dacarbazine for Injection is a sterile, freeze-dried mixture of Dacarbazine and suitable buffers or diluents. It contains NLT 90.0% and NMT 110.0% of the labeled amount of dacarbazine ( $C_6H_{10}N_6O$ ).

**[CAUTION]**—Great care should be taken to prevent inhaling particles of Dacarbazine for Injection and exposing the skin to it.]

### IDENTIFICATION

#### • A.

**Standard solution:** 10 mg/mL each of USP Dacarbazine RS and citric acid in water

**Sample solution:** Equivalent to 10 mg/mL of dacarbazine in water from Dacarbazine for Injection

#### Chromatographic system

(See *Chromatography* (621), *Thin-Layer Chromatography*.)

**Mode:** TLC

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 1  $\mu$ L

**Developing solvent system:** Isopropyl alcohol and 1 N ammonium hydroxide (3:1)

**Spray reagent:** Freshly prepared solution containing 1% of ferric chloride and 1% of potassium ferricyanide (prepared by mixing 5 mL of a 10% aqueous solution of ferric chloride with 5 mL of a 10% aqueous solution of potassium ferricyanide, and diluting with water to 50 mL)

#### Analysis

**Samples:** Standard solution and Sample solution

Develop the chromatogram until the solvent front has moved three-quarters of the length of the plate. Allow the solvent to evaporate. Spray the plate evenly with *Spray reagent*. Dacarbazine appears as an intense blue spot on a light yellow background.

**Acceptance criteria:** The  $R_f$  value of the spot from the Sample solution corresponds to that from the Standard solution.

#### • B.

**Sample solution:** An aqueous solution (1 in 100)

**Analysis:** To 1 mL of the Sample solution in a test tube add a few crystals of periodic acid and 4 drops of methanol. Shake, and after 1 min add 5 mL of a 0.2% acet-



ylacetone reagent solution (prepared by mixing 15.0 g of ammonium acetate, 0.30 mL of glacial acetic acid, and 0.20 mL of acetylacetone in a 100-mL volumetric flask, diluting with water to volume, and mixing). Shake, and place in a water bath maintained at a temperature of 60°.

**Acceptance criteria:** An intense yellow color develops in a few min (presence of mannitol).

• **C.**

**Sample solution:** An aqueous solution (1 in 100)

**Analysis:** To 2 drops of the *Sample solution* in a 15-mL test tube add 10 mL of a solution prepared by mixing 10 mL of acetic anhydride with 30 mL of pyridine.

**Acceptance criteria:** An intense yellow color is produced immediately and, after a few min, becomes red-violet (presence of citric acid).

**ASSAY**

• **PROCEDURE**

**Standard solution:** 3.2 µg/mL of USP Dacarbazine RS in 0.1 N hydrochloric acid

**Sample solution:** Equivalent to 3.2 µg/mL of dacarbazine in 0.1 N hydrochloric acid prepared as follows. Dissolve the contents of NLT 10 containers of Dacarbazine for Injection in 0.1 N hydrochloric acid. Transfer and combine the solutions into a suitable volumetric flask, rinsing as necessary with 0.1 N hydrochloric acid, and dilute with 0.1 N hydrochloric acid to volume.

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** Maximum absorbance at about 323 nm

**Cell:** 1 cm

**Blank:** 0.1 N hydrochloric acid

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dacarbazine ( $C_6H_{10}N_6O$ ) in the portion of Dacarbazine for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Dacarbazine RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of dacarbazine in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–110.0%

- **UNIFORMITY OF DOSAGE UNITS** (905): Meets the requirements

**IMPURITIES**

• **LIMIT OF 2-AZAHYPOXANTHINE**

The *Mobile phase* used in this procedure is corrosive. The system should be rinsed well with methanol after completion of the analysis.

**Mobile phase:** Transfer 2.2 g of docusate sodium to a 1000-mL volumetric flask, dissolve in a mixture of 100 mL of water and 15 mL of glacial acetic acid, and dilute with water to volume. Prepare this solution fresh daily.

**Standard solution:** 0.04 mg/mL of USP Dacarbazine Related Compound B RS in water

**Sample solution:** Constitute the contents of 1 vial of Dacarbazine for Injection, and dilute quantitatively with water to obtain a solution containing 4 mg/mL of dacarbazine.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 1.2 mL/min

**Injection volume:** 20 µL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 2.0% (5 replicate injections)

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of 2-azahypoxanthine in the portion of Dacarbazine for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of 2-azahypoxanthine from the *Sample solution*

$r_S$  = peak response of 2-azahypoxanthine from the *Standard solution*

$C_S$  = concentration of USP Dacarbazine Related Compound B RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of dacarbazine in the *Sample solution*

**Acceptance criteria:** NMT 1.0%

**SPECIFIC TESTS**

• **PH** (791)

**Sample solution:** Equivalent to 10 mg/mL of dacarbazine in water from Dacarbazine for Injection

**Acceptance criteria:** 3.0–4.0

• **WATER DETERMINATION, Method I** (921): NMT 1.5%

• **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 0.52 USP Endotoxin Unit/mg of dacarbazine.

• **STERILITY TESTS** (71): Meets the requirements

• **COMPLETENESS OF SOLUTION:** When dissolved as directed in the labeling, it yields a clear, pale yellow to yellow solution.

• **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests, Completeness and clarity of solutions*.

**ADDITIONAL REQUIREMENTS**

• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers as described in *Packaging and Storage Requirements* (659), *Injection Packaging*, preferably of Type I glass, protected from light.

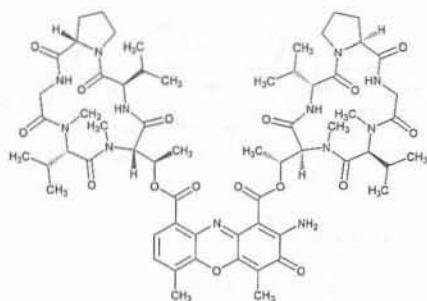
• **LABELING:** Meets requirements in *Labeling* (7), *Labels and Labeling for Injectable Products*

• **USP REFERENCE STANDARDS** (11)  
USP Dacarbazine RS



USP Dacarbazine Related Compound B RS  
2-Azahypoxanthine.  
 $C_4H_3N_5O \cdot H_2O$  155.12  
USP Endotoxin RS

## Dactinomycin



$C_{62}H_{86}N_{12}O_{16}$  1255.42  
Actinomycin D [50-76-0].

### DEFINITION

Dactinomycin contains NLT 950 µg/mg and NMT 1030 µg/mg of dactinomycin ( $C_{62}H_{86}N_{12}O_{16}$ ), calculated on the dried basis.

[CAUTION—Great care should be taken to prevent inhaling particles of Dactinomycin and exposing the skin to it.]

### IDENTIFICATION

#### A. ULTRAVIOLET ABSORPTION (197U)

Standard solution: 25 µg/mL of dactinomycin from USP Dactinomycin RS in methanol

Sample solution: 25 µg/mL in methanol

Analytical wavelengths: 240 and 445 nm

#### Acceptance criteria

Absorptivity: The absorptivity of the *Sample solution* at 445 nm is NLT 95.0% and NMT 103.0% that of the *Standard solution*.

Ratio:  $A_{240}/A_{445}$ , 1.30–1.50, *Sample solution*

#### B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

Mobile phase: Acetonitrile, 0.04 M sodium acetate, and 0.07 M acetic acid (46:25:25). Pass through a filter of 1-µm or finer pore size. The acetonitrile concentration may be varied to provide appropriate chromatographic system performance and a suitable elution time.

Standard solution: 1.2 mg/mL of USP Dactinomycin RS in *Mobile phase*. Use a freshly prepared solution, and protect it from light.

Sample solution: 1.2 mg/mL of Dactinomycin in *Mobile phase*. Use a freshly prepared solution, and protect it from light.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 30-cm; packing L1

Flow rate: 1 mL/min

Injection volume: 20 µL

#### System suitability

Sample: *Standard solution*

[NOTE—The retention time for dactinomycin is about 25 min.]

### Suitability requirements

Relative standard deviation: NMT 1.0%, dactinomycin (3 replicate injections)

### Analysis

Samples: *Standard solution* and *Sample solution*  
Calculate the potency, in µg/mg, of dactinomycin ( $C_{62}H_{86}N_{12}O_{16}$ ) in the Dactinomycin taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Dactinomycin RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

$P$  = potency of dactinomycin in USP Dactinomycin RS (mg/mg)

$F$  = conversion factor, 1000 µg/mg

Acceptance criteria: 950–1030 µg/mg on the dried basis

### SPECIFIC TESTS

#### OPTICAL ROTATION, Specific Rotation (781S)

Sample solution: 1 mg/mL in methanol

Acceptance criteria:  $-293^\circ$  to  $-329^\circ$  ( $t = 20^\circ$ )

#### CRYSTALLINITY (695): Meets the requirements

#### LOSS ON DRYING (731)

Analysis: Dry under vacuum at a pressure not exceeding 5 mm of mercury at  $60^\circ$  for 3 h.

Acceptance criteria: NMT 5.0%

#### BACTERIAL ENDOTOXINS TEST (85): It contains NMT 100 USP Endotoxin Units/mg.

### ADDITIONAL REQUIREMENTS

#### PACKAGING AND STORAGE: Preserve in tight containers, protected from light and excessive heat.

#### USP REFERENCE STANDARDS (11)

USP Dactinomycin RS

USP Endotoxin RS

## Dactinomycin for Injection

### DEFINITION

Dactinomycin for Injection is a sterile mixture of Dactinomycin and Mannitol. It contains NLT 90.0% and NMT 120.0% of the labeled amount of  $C_{62}H_{86}N_{12}O_{16}$ .

[CAUTION—Great care should be taken to prevent inhaling particles of Dactinomycin and exposing the skin to it.]

### IDENTIFICATION

#### A. PROCEDURE

Standard solution: 25 µg/mL of USP Dactinomycin RS in methanol

Sample solution: 25 µg/mL of dactinomycin in methanol

Acceptance criteria: The UV absorption spectrum of the *Sample solution* exhibits maxima and minima at the same wavelengths as the *Standard solution*, concomitantly measured.

Ratio:  $A_{240}/A_{445}$ , 1.30–1.50

#### B. The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

[NOTE—Use freshly prepared *Standard solution* and *Sample solution*, protected from light.]

Mobile phase: Acetonitrile and water (3:2)

Standard solution: 250 µg/mL of USP Dactinomycin RS in *Mobile phase*



**Sample solution:** 250 µg/mL of dactinomycin from Dactinomycin for Injection diluted with *Mobile phase*. Filter, if necessary, to obtain a clear solution. [NOTE—Prepare the solution by adding a suitable aliquot of *Mobile phase* to one container of Dactinomycin for Injection.]

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm × 30-cm; packing L1

**Flow rate:** 2.5 mL/min

**Injection size:** 10 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The retention time for dactinomycin is 6 min.]

#### Suitability requirements

**Column efficiency:** NLT 1200 theoretical plates

**Tailing factor:** NMT 2

**Relative standard deviation:** NMT 3.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of C<sub>62</sub>H<sub>86</sub>N<sub>12</sub>O<sub>16</sub> in the portion of Dactinomycin for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Dactinomycin RS in the *Standard solution* (µg/mL)

$C_U$  = nominal concentration of dactinomycin in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–120.0%

#### SPECIFIC TESTS

- **PH (791):** 5.5–7.5, in the solution constituted as directed in the labeling
- **LOSS ON DRYING (731):** Dry a portion in vacuum at a pressure not exceeding 5 mm of mercury at 60° for 3 h; it loses NMT 4.0% of its weight.
- **OTHER REQUIREMENTS:** It meets the requirements under *Injections and Implanted Drug Products* (1).
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 100.0 USP Endotoxin Units/mg of dactinomycin.
- **STERILITY TESTS (71):** Meets the requirements when tested as directed for *Test for Sterility of the Product to be Examined*, *Membrane Filtration*, each container being constituted aseptically by injecting Sterile Water for Injection through the stopper, and the entire contents of all the containers being collected aseptically with the aid of 200 mL of *Fluid A* before filtering.
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions* and for *Labeling* (7), *Labels and Labeling for Injectable Products*.

#### ADDITIONAL REQUIREMENTS

##### Change to read:

- **PACKAGING AND STORAGE:** Preserve as described in *Packaging and Storage Requirements* (659), *Injection Packaging, Packaging for constitution* (CN 1-May-2017); protect from light.
- **LABELING:** Label it to include the statement "Protect from light."

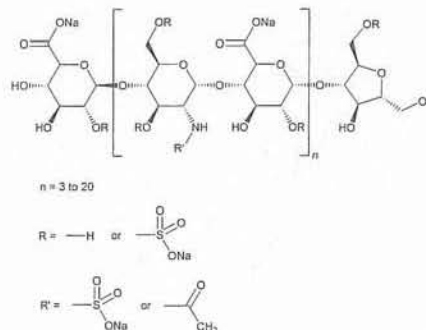
#### • USP REFERENCE STANDARDS (11)

USP Dactinomycin RS

USP Endotoxin RS

## Dalteparin Sodium

[9041-08-1].



#### DEFINITION

Dalteparin Sodium is the sodium salt of a low molecular weight heparin obtained by nitrous acid depolymerization of heparin from porcine intestine or intestinal mucosa. Heparin source material used in the manufacture of Dalteparin Sodium complies with the compendial requirements stated in the Heparin Sodium monograph. Dalteparin Sodium is produced by a validated manufacturing and purification procedure under conditions shown to minimize the presence of species containing the N–NO group. The majority of the components have a 2-O-sulfo-α-L-idopyranosuronic acid structure at the non-reducing end and a 6-O-sulfo-2,5-anhydro-D-mannitol structure at the reducing end of their chains. The weight-average molecular weight ( $M_w$ ) ranges between 5600 Da and 6400 Da, with a characteristic value of about 6000 Da. The percentage of chains lower than molecular weight 3000 Da is NMT 13.0%, and the percentage of chains higher than molecular weight 8000 Da ranges between 15.0% and 25.0%. The degree of sulfation is NLT 1.8/disaccharide unit. The potency is NLT 110 and NMT 210 Anti-Factor Xa International Units (IU)/mg of activity, calculated on the dried basis. The anti-factor IIa activity is NLT 35 IU/mg and NMT 100 IU/mg, calculated on the dried basis. The ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.9 and 3.2.

#### IDENTIFICATION

##### • A. <sup>1</sup>H NMR SPECTRUM

**Standard solution:** Dissolve 15 mg of USP Dalteparin Sodium RS in 0.7 mL of deuterium oxide with deuterated trimethylsilylpropionic (TSP) acid sodium salt. The sample is freeze-dried to remove exchangeable protons. Redissolve the sample and repeat the freeze-drying step twice more before transferring the sample into an NMR tube.

**Sample solution:** Dissolve 15 mg of Dalteparin Sodium in 0.7 mL of deuterium oxide (99.9%) with deuterated TSP. The sample is freeze-dried to remove exchangeable protons. Redissolve the sample and repeat the freeze-drying step twice more before transferring the sample into an NMR tube.



**Instrumental conditions**

(See *Nuclear Magnetic Resonance Spectroscopy* (761).)

**Mode:** NMR, pulsed (Fourier transform)

**Frequency:** NLT 500 MHz for  $^1\text{H}$

**Temperature:** 30°

**System suitability**

**Samples:** *Standard solution* and *Sample solution*

Transfer the *Standard solution* and the *Sample solution* to NMR tubes of 5 mm in diameter. Using a pulsed (Fourier transform) NMR spectrometer operating at NLT 500 MHz for  $^1\text{H}$ , acquire a free induction decay (FID) with NLT 32 scans using a 90° pulse, an acquisition time of NLT 2 s, and at least a 10-s delay. For each sample, an initial short spectrum is collected (1 scan), and the water resonance is then suppressed by selective irradiation during the relaxation delay. Final spectra are recorded over 32 scans. For all samples, the TSP methyl signal should be set to 0.00 ppm. Record the  $^1\text{H}$  NMR spectrum of the *Standard solution*. Collect the  $^1\text{H}$  NMR spectrum with a spectral window of at least 10 to -2 ppm and without spinning. The *Standard solution* shall be run at least daily when the *Sample solution* is being run. All spectra are phased, and linear baseline correction is applied to all spectra before peak identification.

**Suitability requirements**

**Chemical shift:** The TSP methyl signal should be set to 0.00 ppm for all samples.

**Chemical shifts for system suitability:** The ppm values for the methyl group of *N*-acetyl, the H-2 of *N*-sulfo glucosamine, the H-2 of glucuronic acid plus 3-*O*-sulfo glucosamine, the H-1 of iduronic acid, and the H-1 of 3-*O*-sulfo glucosamine of dalteparin in the *Standard solution* are present at 2.05, 3.28, 3.39, 5.01, and 5.51, respectively. Two additional signals, corresponding to the H-1 of the 2-*O*-sulfo iduronic acid linked to the terminal 2, 5-anhydromannitol and the H-1 of 2-*O*-sulfo iduronic acid are located at 5.18–5.22 ppm. The ppm values of these signals do not differ by more than  $\pm 0.03$  ppm, *Standard solution*. [NOTE—Depending on specific sample makeup and instrument parameters, including the field strength of the NMR instrument, the two signals associated with the H-1 of 2-*O*-sulfo iduronic acid at 5.18–5.22 ppm may appear well separated or as a main signal with a shoulder.]

**Analysis**

**Sample:** *Sample solution*

Record the  $^1\text{H}$  NMR spectra of the *Sample solution*.

**Acceptance criteria:** The ppm values for the methyl group of *N*-acetyl, the H-2 of *N*-sulfo glucosamine, the H-2 of glucuronic acid plus 3-*O*-sulfo glucosamine, the H-1 of iduronic acid and the H-1 of the 2-*O*-sulfo iduronic acid linked to the terminal anhydromannitol, the H-1 of 2-*O*-sulfo iduronic acid and the H-1 of 3-*O*-sulfo glucosamine of dalteparin in the *Sample solution* are present at 2.05, 3.28, 3.39, 5.01, 5.18–5.22, and 5.51, respectively. The ppm values of these signals do not differ by more than  $\pm 0.03$  ppm.

• **B. MOLECULAR WEIGHT DISTRIBUTION AND WEIGHT-AVERAGE MOLECULAR WEIGHT**

(See *Low Molecular Weight Heparin Molecular Weight Determinations* (209).)

**Acceptance criteria:** The weight-average molecular weight ( $M_w$ ) ranges between 5600 Da and 6400 Da, with a characteristic value of about 6000 Da. The percentage of chains lower than the molecular weight 3000 Da ( $M_{3000}$ ) is NMT 13.0%, and the percentage of chains higher than the molecular weight 8000 Da ( $M_{8000}$ ) ranges between 15.0% and 25.0%.

• **C. ANTI-FACTOR Xa TO ANTI-FACTOR IIa RATIO**

(See *Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins* (208), *Anti-Factor Xa and Anti-Factor IIa Assays for Low Molecular Weight Heparins*.)

**Acceptance criteria:** The ratio of the numerical value of the anti-factor Xa activity, in Anti-Factor Xa IU/mg, to the numerical value of the anti-factor IIa activity, in Anti-Factor IIa IU/mg, as determined by the *Anti-Factor Xa Activity* and *Anti-Factor IIa Activity* assays, is NLT 1.9 and NMT 3.2, respectively.

- **D. IDENTIFICATION TESTS—GENERAL:** Meets the requirements for *Sodium Content*

**ASSAY**

- **ANTI-FACTOR Xa ACTIVITY**

(See *Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins* (208), *Anti-Factor Xa and Anti-Factor IIa Assays for Low Molecular Weight Heparins*, *Anti-Factor Xa Activity for Low Molecular Weight Heparin*.)

**Analysis:** Proceed as directed in the chapter.

**Acceptance criteria:** The potency is NLT 110 and NMT 210 Anti-Factor Xa IU/mg on the dried basis.

**OTHER COMPONENTS**

- **NITROGEN DETERMINATION, Method II (461):** 1.5%–2.5% on the dried basis

- **SODIUM CONTENT**

**Cesium chloride solution:** 1.27 mg/mL of cesium chloride in 0.1 M hydrochloric acid

**Standard solution A:** 0.0025% of sodium chloride in *Cesium chloride solution*

**Standard solution B:** 0.0050% of sodium chloride in *Cesium chloride solution*

**Standard solution C:** 0.0075% of sodium chloride in *Cesium chloride solution*

**Sample solution:** Transfer 50.0 mg of Dalteparin Sodium to a 100-mL volumetric flask, and dissolve in and dilute with *Cesium chloride solution* to volume.

**Analysis**

**Samples:** *Cesium chloride solution*, *Standard solution A*, *Standard solution B*, *Standard solution C*, and *Sample solution*

Concomitantly determine the absorbances of the *Cesium chloride solution* (blank), the *Sample solution*, and the *Standard solutions* at 330.3 nm, using a sodium hollow-cathode lamp and an air-acetylene flame. Using the absorbances of *Standard solutions A*, *B*, and *C*, determine the sodium content in the *Sample solution* after an appropriate blank correction.

**Acceptance criteria:** 10.5%–13.5% on the dried basis

**IMPURITIES**

- **LIMIT OF NITRITES**

**Mobile phase:** Dissolve 13.6 g of sodium acetate trihydrate in 900 mL of water in a 1000-mL volumetric flask. Adjust with orthophosphoric acid to a pH of 4.3, and dilute with water to 1000 mL. Filter through a 0.45- $\mu\text{m}$  membrane.

**Nitrite stock standard solution:** Dissolve 0.075 g of sodium nitrite in a 1000-mL volumetric flask with carbon dioxide-free water (0.05 g/L of nitrite).

**Nitrite standard solution:** Dilute 1 mL of *Nitrite stock standard solution* in a 100-mL volumetric flask with carbon dioxide-free water (500 ng/mL of nitrite).

**Calibration standard solutions:** Dilute *Nitrite standard solution* in carbon dioxide-free water to prepare four solutions with the final nitrite concentrations of 2.5, 5, 15, and 25 ng/mL.

**Sample solution:** Weigh 80.0 mg of Dalteparin Sodium into a 20-mL volumetric flask, and dissolve in carbon dioxide-free water.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** Electrochemical detector containing a working electrode (glassy carbon type) with the potential of +1.00 V against a silver-silver chloride reference electrode



**Column:** 3-mm × 15-cm; 5-μm packing L92

**Column temperature:** 30 ± 5°

**Column regeneration:** 1 M sodium chloride (NaCl) at 0.5 mL/min for about 1 h. After regeneration, wash the column with water and re-equilibrate with *Mobile phase*.

**Flow rate:** 0.5 mL/min

**Injection volume:** 25 μL

**Run time:** 10 min

#### System suitability

**Samples:** *Calibration standard solutions* and *Sample solution*

#### Suitability requirements

**Column efficiency:** NLT 4000 theoretical plates for the nitrite peak for all *Calibration solutions* and *Sample solution* runs

**Tailing factor:** Between 0.8 and 1.2 for all *Calibration solutions* and *Sample solution* runs

**Relative standard deviation:** Inject *Calibration standard solutions* with 25 ng/mL concentration at least six times. Calculate the relative standard deviation % (%RSD) of the nitrite peak areas of the last six injections. The %RSD is NMT 2%.

#### Analysis

**Samples:** *Calibration standard solutions* and *Sample solution*

Plot the areas of the nitrite peaks from the chromatograms of the *Calibration standard solutions* against respective concentrations of nitrite. Draw a best-fit regression line through the points. The correlation coefficient is NLT 0.995. Calculate the concentration of nitrite from the areas of the nitrite peak in the chromatogram of the *Sample solution*.

**Acceptance criteria:** NMT 5 ppm

#### • BORON

[NOTE—Use only plastic labware, avoid glass.]

**Blank:** 1% (v/v) solution of nitric acid in water

**Calibration solution:** Prepare a 11.4-μg/mL solution of USP Boric Acid RS in the *Blank*.

**Standard solution A:** Dissolve 0.2500 g of USP Low Molecular Weight Heparin for Boron Analysis RS in about 2 mL of water, add 100 μL of nitric acid, and dilute with the *Blank* to 10.00 mL.

**Standard solution B:** Dissolve 0.2500 g of USP Low Molecular Weight Heparin for Boron Analysis RS in about 2 mL of *Blank*, add 10 μL of a 5.7-mg/mL solution of USP Boric Acid RS, and dilute with the *Blank* to 10.00 mL. This solution contains 1 μg/mL of boron.

**Sample solution:** Dissolve 0.2500 g of Dalteparin Sodium in about 2 mL of water, add 100 μL of nitric acid, and dilute with the *Blank* to 10.00 mL.

#### Analysis

**Samples:** *Blank*, *Calibration solution*, *Standard solution A*, *Standard solution B*, and *Sample solution*

Boron is determined by measurement of the emission from inductively coupled plasma (ICP) at 249.733 nm or a suitable wavelength. Use an appropriate apparatus with settings that have been optimized as directed by the manufacturer.

Calculate the content of boron in Dalteparin Sodium using the following correction factor:

$$F = (r_{SB} - r_{SA}) \times 2 / (r_C - r_B)$$

$r_{SB}$  = response of boron from *Standard solution B*

$r_{SA}$  = response of boron from *Standard solution A*

$r_C$  = response of boron from the *Calibration solution*

$r_B$  = response of boron from the *Blank*

**Acceptance criteria:** NMT 1 ppm

### SPECIFIC TESTS

#### • ANTI-FACTOR IIA ACTIVITY

(See *Anti-Factor Xa and Anti-Factor Ila Assays for Unfractionated and Low Molecular Weight Heparins*, <208>, *Anti-Factor Xa and Anti-Factor Ila Assays for Low Molecular*

*Weight Heparins, Anti-Factor Ila Activity for Low Molecular Weight Heparins*.)

**Acceptance criteria:** NLT 35 and NMT 100 Anti-Factor Ila IU/mg on the dried basis

#### • MOLAR RATIO OF SULFATE TO CARBOXYLATE

**Mobile phase:** Carbon dioxide-free water

**Sample solution:** 50 mg of Dalteparin Sodium in 10 mL of carbon dioxide-free water

#### Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

**Mode:** LC

**Detector:** Ion

**Column:** Two columns: one 1.5-cm × 2.5-cm column, packed with an anion-exchange resin L64 packing, and one 1.5-cm × 7.5-cm column, packed with a cation-exchange resin L65 packing.<sup>1</sup> The outlet of the anion-exchange column is connected to the inlet of the cation-exchange column.

**Flow rate:** 1 mL/min

#### Analysis

**Sample:** *Sample solution*

[NOTE—Regenerate the anion-exchange column and the cation-exchange column with 1 N sodium hydroxide and 1 N hydrochloric acid, respectively, between two injections.]

With the valve in the inject position, inject the *Sample solution* into the anion-exchange column, and collect the eluate from the cation-exchange column in a beaker at the outlet until the ion detector reading returns to the baseline value. Quantitatively transfer the eluate to a titration vessel containing a magnetic stirring bar, and dilute with carbon dioxide-free water to about 60 mL. Position the titration vessel on a magnetic stirrer, and immerse the electrodes. Note the initial conductivity reading, and titrate with approximately 0.1 N sodium hydroxide added in 100-μL portions. [NOTE—Prepare the sodium hydroxide solution in carbon dioxide-free water.] Record the buret reading and the conductivity meter reading after each addition of the sodium hydroxide solution.

Plot the conductivity measurements on the y-axis against the volumes of sodium hydroxide added on the x-axis. The graph will have three linear sections—an initial downward slope, a middle slight rise, and a final rise. For each of these sections, draw the best-fit straight lines using linear regression analysis. At the points where the first and second straight lines intersect and where the second and third lines intersect, draw perpendiculars to the x-axis to determine the volumes of sodium hydroxide taken up by the sample at those points. The point where the first and second lines intersect corresponds to the volume of sodium hydroxide taken up by the sulfate groups ( $V_S$ ). The point where the second and third lines intersect corresponds to the volume of sodium hydroxide consumed by the sulfate and the carboxylate groups together ( $V_T$ ).

Calculate the molar ratio of sulfate to carboxylate:

$$\text{Result} = V_S / (V_T - V_S)$$

**Acceptance criteria:** The molar ratio of sulfate to carboxylate is NLT 1.8.

• **PH (791):** 5.5–8.0 for a 1.0% solution in water

• **LOSS ON DRYING (731)**

**Sample:** 1 g

**Analysis:** Dry the *Sample* under vacuum at 70° for 6 h.

**Acceptance criteria:** NMT 10%

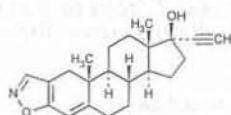
• **BACTERIAL ENDOTOXINS TEST (85):** It contains NMT 0.01 USP Endotoxin Unit/IU of anti-factor Xa activity.

<sup>1</sup> The procedure is based on analyses performed with two columns: one 1.5-cm × 2.5-cm packed with anion-exchange resin Dowex 1X8 (200–400 mesh) and the other 1.5-cm × 7.5-cm packed with cation-exchange resin Dowex 50WX2 (100–200 mesh).



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers, and store below 40°, preferably at room temperature.
- **LABELING:** Label to state the number of Anti-factor X<sub>a</sub> International Units of activity per mg.
- **USP REFERENCE STANDARDS** (11)
  - USP Boric Acid RS
  - USP Dalteparin Sodium RS
  - USP Endotoxin RS
  - USP Low Molecular Weight Heparin for Bioassays RS
  - USP Low Molecular Weight Heparin for Boron Analysis RS
  - USP Low Molecular Weight Heparin Molecular Weight Calibrant RS

**Danazol**C<sub>22</sub>H<sub>27</sub>NO<sub>2</sub> 337.46

Pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol, (17 $\alpha$ )-  
17 $\alpha$ -Pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol  
[17230-88-5].

» Danazol contains not less than 97.0 percent and not more than 102.0 percent of C<sub>22</sub>H<sub>27</sub>NO<sub>2</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Danazol RS

**Identification**—

A: Infrared Absorption (197K).

B: Ultraviolet Absorption (197U)—

Solution: prepared as directed in the Assay.

**Specific rotation** (781S): between +21° and +27°.

Test solution: 10 mg per mL, in chloroform.

**Loss on drying** (731)—Dry it at a pressure not exceeding 5 mm of mercury at 60° to constant weight: it loses not more than 2.0% of its weight.

**Chromatographic purity**—

Solvent—Prepare a mixture of chloroform and methanol (9:1).

Standard solutions—Dissolve an accurately weighed quantity of USP Danazol RS in Solvent to obtain a solution having a known concentration of 1 mg per mL. Dilute quantitatively with Solvent to obtain Standard solutions having the following compositions:

Standard solution	Dilution	Concentration ( $\mu$ g RS per mL)	Percentage (%, for comparison with test specimen)
A	(1 in 2)	500	1.0
B	(1 in 4)	250	0.5
C	(1 in 10)	100	0.2
D	(1 in 20)	50	0.1

Test solution—Dissolve an accurately weighed quantity of Danazol in Solvent to obtain a solution containing 50 mg per mL.

**Procedure**—Apply separately 5  $\mu$ L of the Test solution and 5  $\mu$ L of each Standard solution to a suitable thin-layer chromatographic plate (see Chromatography (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Position the plate in a chromatographic chamber and develop the chromatograms in a solvent system consisting of a mixture of cyclohexane and ethyl acetate (7:3) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate in warm, circulating air. Examine the plate under short-wave-length UV light. Expose the plate to iodine vapors for 5 minutes. Compare the intensities of any secondary spots observed in the chromatogram of the Test solution with those of the principal spots in the chromatograms of the Standard solutions: the sum of the intensities of secondary spots obtained from the Test solution corresponds to not more than 1.0% of related compounds, with no single impurity corresponding to more than 0.5%.

**Assay**—Dissolve about 100 mg of Danazol, accurately weighed and previously dried, in about 50 mL of alcohol in a 100-mL volumetric flask, swirl until dissolved, dilute with alcohol to volume, and mix. Transfer 2.0 mL of this solution to a 100-mL volumetric flask, dilute with alcohol to volume, and mix. Similarly, dissolve an accurately weighed quantity of USP Danazol RS in alcohol to obtain a Standard solution having a known concentration of about 20  $\mu$ g per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 285 nm, using alcohol as the blank. Calculate the quantity, in mg, of C<sub>22</sub>H<sub>27</sub>NO<sub>2</sub> in the portion of Danazol taken by the formula:

$$5C(A_U / A_S)$$

in which C is the concentration, in  $\mu$ g per mL, of USP Danazol RS in the Standard solution; and A<sub>U</sub> and A<sub>S</sub> are the absorbances of the solution of Danazol and the Standard solution, respectively.

**Danazol Capsules**

» Danazol Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>22</sub>H<sub>27</sub>NO<sub>2</sub>.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Danazol RS

**Identification**—Shake the contents of a sufficient number of Capsules, equivalent to about 50 mg of Danazol, with 50 mL of chloroform, and filter. Evaporate the filtrate on a steam bath with the aid of a stream of nitrogen to dryness: the IR absorption spectrum of a potassium bromide dispersion of the residue, previously dried, exhibits maxima at the same wavelengths as that of a similar preparation of USP Danazol RS.

**Dissolution** (711)—

Medium: 0.75% sodium lauryl sulfate solution; 900 mL.

Apparatus 2: 75 rpm.

Time: 30 minutes.

**Procedure**—Determine the amount of C<sub>22</sub>H<sub>27</sub>NO<sub>2</sub> dissolved as follows. Remove an aliquot from the solution under test at a point midway between the stirring shaft and the wall of the vessel and approximately midway in depth. Measure the amount in solution in filtered portions of the Dissolution Medium, suitably diluted with the Dissolution Medium, at the wavelength of maximum absorbance at about



286 nm, with a suitable spectrophotometer, in comparison with a solution of known concentration of USP Danazol RS prepared as follows. Transfer 10 mg of USP Danazol RS, accurately weighed, to a 10-mL volumetric flask, and dissolve in isopropyl alcohol. Transfer 2.0 mL to a 100-mL volumetric flask, dilute with *Dissolution Medium* to volume, and mix.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $C_{22}H_{27}NO_2$  is dissolved in 30 minutes.

**Uniformity of dosage units** (905): meet the requirements.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of acetonitrile, methanol, and water (4:3:3). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Danazol RS in *Mobile phase* to obtain a solution having a known concentration of about 0.2 mg per mL.

**Assay preparation**—Accurately weigh the contents of not less than 20 Capsules. Mix the contents, and transfer an accurately weighed portion of the powder, equivalent to about 100 mg of danazol, to a 100-mL volumetric flask. Add about 50 mL of *Mobile phase*, and shake by mechanical means for about 10 minutes. Dilute with *Mobile phase* to volume, mix, and filter, discarding the first 5 mL of the filtrate. Pipet 5 mL of the filtrate into a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Filter a portion of this solution through a 0.45- $\mu$ m porosity filter, discarding the first 5 mL of the filtrate.

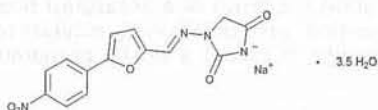
**Chromatographic system** (see *System Suitability* under *Chromatography* (621))—The liquid chromatograph is equipped with a 270-nm detector and a 3.9-mm  $\times$  15-cm column that contains 4- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of danazol in the portion of Capsules taken by the formula:

$$500C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Danazol RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dantrolene Sodium



$C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$  399.29  
2,4-Imidazolidinedione, 1-[[[5-(4-nitrophenyl)-2-furanyl-1]methylene]amino]-, sodium salt, hydrate (2:7);  
1-[[[5-(p-Nitrophenyl)furfurylidene]amino]hydantoin sodium salt hydrate [24868-20-0].

#### DEFINITION

Dantrolene Sodium contains NLT 90.0% and NMT 96.0% of dantrolene ( $C_{14}H_9N_4O_5$ ), the free acid form of Dantrolene Sodium, calculated on the anhydrous basis.

#### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL** (191), *Sodium*  
**Sample solution:** To 0.1 g of Dantrolene Sodium, add 20 mL of water and 2 drops of acetic acid, shake well, and pass the resulting solution through a suitable filter. Use 2 mL of the filtrate.  
**Analysis**  
**Sample:** *Sample solution*  
**Acceptance criteria:** Meets the requirements

- **D.**  
**Solution A:** Dissolve 2.7 g of methoxyphenylacetic acid in 6 mL of tetramethylammonium hydroxide solution, and add 20 mL of dehydrated alcohol.

**Solution B:** 158 mg/mL of ammonium carbonate in water

**Sample solution:** To 0.1 g of Dantrolene Sodium, add 20 mL of water and 2 drops of acetic acid, shake well, and pass the resulting solution through a suitable filter. Use the filtrate.

#### Analysis

**Sample:** *Sample solution*

**Part 1:** To 0.5 mL of the *Sample solution* in a suitable container, add 1.5 mL of *Solution A*, and cool in ice water for 30 min.

**Part 2:** Transfer the container from *Part 1* to a water bath at 20°, and stir for 5 min.

**Part 3:** Add 1 mL of ammonia TS to the container from *Part 2*.

**Part 4:** Add 1 mL of *Solution B* to the container from *Part 3*.

**Acceptance criteria:** The requirements for *Part 1*, *Part 2*, *Part 3*, and *Part 4* must all be met.

**Part 1:** A voluminous, white, crystalline precipitate is formed.

**Part 2:** The precipitate does not disappear.

**Part 3:** The precipitate dissolves completely.

**Part 4:** No precipitate is formed.

#### ASSAY

##### PROCEDURE

**Buffer:** Dissolve 3.85 g of ammonium acetate in 1.0 L of water; adjusted with glacial acetic acid to a pH of  $4.5 \pm 0.1$ .

**Solution A:** Acetonitrile, *Buffer*, and water (10:20:70)

**Solution B:** Acetonitrile and *Buffer* (80:20)

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	60	40
20	10	90
25	10	90
25.1	90	10
35	90	10

**Diluent:** Acetonitrile and water (50:50)

**System suitability stock solution A:** 1.25 mg/mL of USP Dantrolene Sodium RS prepared as follows. Transfer a suitable amount of USP Dantrolene Sodium RS to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

**System suitability stock solution B:** 0.125 mg/mL each of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS prepared as follows. Transfer suitable amounts of USP Dantrolene Re-



lated Compound B RS and USP Dantrolene Related Compound C RS to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

**System suitability solution:** 125 µg/mL of USP Dantrolene Sodium RS from *System suitability stock solution A* and 2.5 µg/mL each of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS from *System suitability stock solution B* in *Diluent*

**Standard stock solution:** 1.0 mg/mL of USP Dantrolene RS prepared as follows. Transfer a suitable amount of USP Dantrolene RS to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

**Standard solution:** 100 µg/mL of USP Dantrolene RS from *Standard stock solution* in *Diluent*

**Sample stock solution:** 1.25 mg/mL of Dantrolene Sodium prepared as follows. Transfer a suitable amount of Dantrolene Sodium to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

**Sample solution:** 125 µg/mL of Dantrolene Sodium from *Sample stock solution* in *Diluent*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 365 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 2 mL/min

**Injection volume:** 20 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for dantrolene related compound B, dantrolene, and dantrolene related compound C are 0.68, 1.0, and 1.24, respectively.]

#### Suitability requirements

**Resolution:** NLT 8 between dantrolene related compound C and dantrolene, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of dantrolene (C<sub>14</sub>H<sub>10</sub>N<sub>4</sub>O<sub>5</sub>) in the portion of Dantrolene Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of dantrolene from the *Sample solution*

$r_S$  = peak response of dantrolene from the *Standard solution*

$C_S$  = concentration of USP Dantrolene RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Dantrolene Sodium in the *Sample solution* (µg/mL)

**Acceptance criteria:** 90.0%–96.0% on the anhydrous basis

#### IMPURITIES

##### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 20 ppm • (Official 1-Jan-2018)

- **LIMIT OF DANTROLENE RELATED COMPOUND A**

**Mobile phase:** Acetonitrile and water (80:20)

**Standard stock solution:** 17.5 µg/mL of USP Dantrolene Related Compound A RS and 50 µg/mL of USP Dantrolene Sodium RS in dimethylformamide

**Standard solution:** 0.35 µg/mL of USP Dantrolene Related Compound A RS and 1 µg/mL of USP Dantrolene Sodium RS from *Standard stock solution* in acetonitrile

**Sample stock solution:** 1.25 mg/mL of Dantrolene Sodium prepared as follows. Transfer a suitable amount of Dantrolene Sodium to an appropriate volumetric flask. Dissolve in 5% of the total flask volume of dimethylformamide. Add 5% of the total flask volume of glacial acetic acid, and dilute with acetone to volume.

**Sample solution:** 175 µg/mL of Dantrolene Sodium from *Sample stock solution* in acetonitrile

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 365 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L1

**Flow rate:** 1 mL/min

**Injection volume:** 20 µL

#### System suitability

**Sample:** *Standard solution*

[NOTE—The dantrolene peak elutes at void volume at approximately 1.5 min.]

#### Suitability requirements

**Tailing factor:** NMT 1.5 for dantrolene related compound A

**Relative standard deviation:** NMT 5% for dantrolene related compound A

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of dantrolene related compound A in the portion of Dantrolene Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of dantrolene related compound A from the *Sample solution*

$r_S$  = peak response of dantrolene related compound A from the *Standard solution*

$C_S$  = concentration of USP Dantrolene Related Compound A RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Dantrolene Sodium in the *Sample solution* (µg/mL)

**Acceptance criteria:** NMT 0.15%

- **ORGANIC IMPURITIES**

**Mobile phase, Diluent, System suitability stock solution B, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution A:** Use the *Standard solution* from the *Assay*.

**Standard solution B:** 0.25 µg/mL each of USP Dantrolene Related Compound B RS and USP Dantrolene Related Compound C RS from *System suitability stock solution B* in *Diluent*

#### System suitability

**Samples:** *System suitability solution* and *Standard solution A*

[NOTE—The relative retention times for dantrolene related compound B, dantrolene, and dantrolene related compound C are 0.68, 1.0, and 1.24, respectively.]

#### Suitability requirements

**Resolution:** NLT 8 between dantrolene related compound C and dantrolene, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution A*

**Relative standard deviation:** NMT 1.0%, *Standard solution A*

#### Analysis

**Samples:** *Sample solution* and *Standard solution B*

Calculate the percentage of dantrolene related compound B and dantrolene related compound C in the portion of Dantrolene Sodium taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$



- $r_U$  = peak response of dantrolene related compound B or dantrolene related compound C from the *Sample solution*
- $r_S$  = peak response of dantrolene related compound B or dantrolene related compound C from *Standard solution B*
- $C_S$  = concentration of USP Dantrolene Related Compound B RS or USP Dantrolene Related Compound C RS in *Standard solution B* ( $\mu\text{g/mL}$ )
- $C_U$  = concentration of Dantrolene Sodium in the *Sample solution* ( $\mu\text{g/mL}$ )

**Acceptance criteria**

- Dantrolene related compound B: NMT 0.50%
- Dantrolene related compound C: NMT 0.30%

**SPECIFIC TESTS**

- **WATER DETERMINATION** (921), *Method 1a*: 14.5%–17.0%

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers. Store at room temperature.

• **USP REFERENCE STANDARDS** (11)

USP Dantrolene RS

1-((5-(4-Nitrophenyl)furan-2-yl)methylene)amino)imidazolidine-2,4-dione.

$\text{C}_{14}\text{H}_{10}\text{N}_4\text{O}_5$  314.25

USP Dantrolene Sodium RS

USP Dantrolene Related Compound A RS

1,2-Bis[[5-(4-nitrophenyl)furan-2-yl]methylene]hydrazine;

Also known as 5-(4-Nitrophenyl)furaldehyde azine.

$\text{C}_{22}\text{H}_{14}\text{N}_4\text{O}_6$  430.38

USP Dantrolene Related Compound B RS

N-Carbamoyl-N-((5-(4-nitrophenyl)furan-2-yl)methylene)amino)glycine;

Also known as 5-(4-Nitrophenyl)-2-furaldehyde-2-carboxymethyl semicarbazone.

$\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}_6$  332.27

USP Dantrolene Related Compound C RS

5-(4-Nitrophenyl)furan-2-carbaldehyde.

$\text{C}_{11}\text{H}_7\text{NO}_4$  217.18

**Apparatus 1**: 100 rpm.

**Time**: 40 minutes.

Determine the amount of  $\text{C}_{14}\text{H}_9\text{N}_4\text{NaO}_5 \cdot 3\frac{1}{2}\text{H}_2\text{O}$  dissolved by employing the following method.

**Standard solution 1** (for Capsules labeled to contain 100 mg)—Transfer 25 mg of USP Dantrolene RS, accurately weighed, to a 250-mL volumetric flask. Dissolve in 5.0 mL of dimethylformamide. Add 200 mL of *Medium* and 10.0 mL of 0.1 N potassium hydroxide. Mix, dilute with *Medium* to volume, and mix. Pass through a 0.45- $\mu\text{m}$  polytetrafluoroethylene (PTFE) filter, previously wetted with a few drops of isopropyl alcohol, discarding the first 5 mL.

**Standard solution 2** (for Capsules labeled to contain 50 mg)—Transfer 25.0 mL of *Standard solution 1* to a 50-mL volumetric flask containing 0.5 mL of 0.1 N potassium hydroxide. Dilute with *Medium* to volume, and mix. Pass through a 0.45- $\mu\text{m}$  PTFE filter, previously wetted with a few drops of isopropyl alcohol, discarding the first 5 mL.

**Standard solution 3** (for Capsules labeled to contain 25 mg)—Transfer 25.0 mL of *Standard solution 1* to a 100-mL volumetric flask containing 1.0 mL of 0.1 N potassium hydroxide. Dilute with *Medium* to volume, and mix. Pass through a 0.45- $\mu\text{m}$  PTFE filter, previously wetted with a few drops of isopropyl alcohol, discarding the first 5 mL.

**Test solution**—Withdraw 10 mL of the solution under test. Pass through a 0.45- $\mu\text{m}$  PTFE filter, previously wetted with a few drops of isopropyl alcohol. Discard the first 5 mL. Collect the filtered solution in a tube that contains 1 drop of 1 N potassium hydroxide, and mix.

**System suitability**—[NOTE—All absorbance values should be obtained on solutions within 2 hours of their preparation.] Using a 0.1-cm cell, measure the absorbance of the *Medium*, using water as the blank, and measure the absorbance of each of the three *Standard solutions* using *Medium* as the blank, at the wavelength of maximum absorbance at about 395 nm. The system is considered suitable for use if the following criteria are met: the absorbance of the *Medium* is less than 10% of the absorbance of *Standard solution 1*; the absorbance of *Standard solution 2* is between 0.3 and 0.5; and the ratio of the absorbance of *Standard solution 1* to that of *Standard solution 3* is  $4.00 \pm 0.10$ .

Determine the amount of  $\text{C}_{14}\text{H}_9\text{N}_4\text{NaO}_5 \cdot 3\frac{1}{2}\text{H}_2\text{O}$  dissolved by measuring the absorbance of the *Test solution* at the wavelength of maximum absorbance at about 395 nm in comparison with the appropriate *Standard solution*, using a 0.1-cm cell and *Medium* as the blank. All absorbance values are obtained on solutions within 2 hours of their preparation. Calculate the percentage of  $\text{C}_{14}\text{H}_9\text{N}_4\text{NaO}_5 \cdot 3\frac{1}{2}\text{H}_2\text{O}$  dissolved by the formula:

$$\frac{A_U \times C_S \times 900 \times 100}{A_S \times 0.79186 \times LC}$$

in which  $A_U$  and  $A_S$  are the absorbances obtained from the *Test solution* and the *Standard solution*, respectively;  $C_S$  is the concentration, in mg per mL, of dantrolene in the *Standard solution*; 900 is the volume, in mL, of *Medium*; 100 is the conversion factor to percentage; 0.79186 is the correction for water of hydration and sodium contained in the dantrolene sodium monohydrate form of the drug, assuming that the bulk drug contains 15% of water and 6.84% of sodium; and  $LC$  is the Capsule label claim, in mg.

**Tolerances**—Not less than 75% (Q) of the labeled amount of  $\text{C}_{14}\text{H}_9\text{N}_4\text{NaO}_5 \cdot 3\frac{1}{2}\text{H}_2\text{O}$  is dissolved in 40 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Related compounds**—

**Diluent, Solution A, Solution B, Mobile phase, and Chromatographic system**—Proceed as directed in the Assay.

**Standard solution**—Transfer 5 mg, accurately weighed, of USP Dantrolene Related Compound B RS into a 50-mL volu-

## Dantrolene Sodium Capsules

» Dantrolene Sodium Capsules contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dantrolene sodium ( $\text{C}_{14}\text{H}_9\text{N}_4\text{NaO}_5 \cdot 3\frac{1}{2}\text{H}_2\text{O}$ ).

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Dantrolene RS

USP Dantrolene Related Compound B RS

5-(4-Nitrophenyl)-2-furaldehyde-2-carboxymethyl semicarbazone.

$\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}_6$

USP Dantrolene Sodium RS

**Identification**—

**A**: *Infrared Absorption* (197K).

**B**: The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the Assay.

**Dissolution** (711)—

**Medium**: 0.5% methylbenzethonium chloride in water, pH 6.8 (adjusted with 0.1 N potassium hydroxide or 0.1 N hydrochloric acid); 900 mL, deaerated.



metric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume. The final concentration is about 0.1 mg per mL. Quantitatively dilute this solution with *Diluent* to obtain a solution having a known concentration of about 0.0005 mg per mL of dantrolene related compound B.

**Test solution**—Use the *Assay preparation*.

**Procedure**—Inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of dantrolene related compound B in the portion of Capsules taken by the formula:

$$100(r_U/r_S)(C_S/C_T)$$

in which  $r_U$  is the individual peak response for dantrolene related compound B obtained from the *Test solution*;  $r_S$  is the response of the corresponding peak in the *Standard solution*;  $C_S$  is the concentration, in mg per mL, of dantrolene related compound B in the *Standard solution*; and  $C_T$  is the concentration, in mg per mL, of dantrolene sodium in the *Test solution*; not more than 2% of dantrolene related compound B is found.

#### Assay—

**Diluent**—Prepare a solution of acetonitrile and water (70:30).

**Buffer solution**—Dissolve 3.3 g of ammonium acetate in 1 L of water.

**Solution A**—Prepare a filtered and degassed mixture of *Buffer solution*, acetonitrile, and glacial acetic acid (120:76:7).

**Solution B**—Prepare a filtered and degassed mixture of acetonitrile and water (70:30).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B*, as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**System suitability solution**—Use the *Standard solution*, prepared as directed in the test for *Related compounds*.

**Standard preparation**—Transfer 40 mg, accurately weighed, of USP Dantrolene RS to a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume. The final concentration is about 0.8 mg per mL. Quantitatively dilute this solution with *Diluent* to obtain a solution having a known concentration of about 0.08 mg per mL of dantrolene.

**Assay preparation**—Mix the combined contents of not fewer than 20 Capsules, and transfer an accurately weighed portion, equivalent to the average weight of one Capsule, to a 50-mL volumetric flask. Add 10 mL of dimethylformamide, and sonicate for 15 minutes to dissolve. Add 5 mL of glacial acetic acid, and dilute with acetone to volume. Quantitatively dilute this solution with *Diluent* to obtain a solution having 0.1 mg per mL of dantrolene sodium, and pass through a 0.45- $\mu$ m nylon filter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 365-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–8	100	0	isocratic
8–8.1	100 $\rightarrow$ 0	0 $\rightarrow$ 100	linear gradient
8.1–13	0	100	isocratic
13–13.1	0 $\rightarrow$ 100	100 $\rightarrow$ 0	linear gradient
13.1–20	100	0	re-equilibration

Separately inject the *System suitability solution* and the *Standard preparation* into the chromatograph, record the chromatograms, and measure the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections for dantrolene is not more than 1.0%. [NOTE—For the purpose of peak identification, the approximate relative retention times are 0.68 for dantrolene related compound B and 1.0 for dantrolene.]

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the dantrolene peaks. Calculate the percentage of dantrolene sodium ( $C_{14}H_9N_4NaO_5 \cdot 3^{1/2}H_2O$ ) in the portion of Capsules taken by the formula:

$$100(399.29/314.25)(r_U/r_S)(C_S/C_U)$$

in which 399.29 is the molecular weight of dantrolene sodium; 314.25 is the molecular weight of dantrolene;  $r_U$  and  $r_S$  are the peak responses for dantrolene obtained from the *Assay preparation* and the *Standard preparation*, respectively;  $C_S$  is the concentration, in mg per mL, of dantrolene in the *Standard preparation*; and  $C_U$  is the concentration, in mg per mL, of dantrolene sodium ( $C_{14}H_9N_4NaO_5 \cdot 3^{1/2}H_2O$ ) in the *Assay preparation*.

## Dantrolene Sodium for Injection

» Dantrolene Sodium for Injection is a sterile, non-pyrogenic, lyophilized formulation containing Dantrolene Sodium, and one or more suitable buffering or sequestering agents. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{14}H_9N_4NaO_5 \cdot 3^{1/2}H_2O$ .

**Packaging and storage**—Preserve in tight containers. Store at controlled room temperature, protected from light.

#### USP Reference standards (11)—

USP Dantrolene RS

USP Dantrolene Related Compound B RS

5-(4-Nitrophenyl)-2-furaldehyde-2-carboxymethyl semicarbazone.

$C_{14}H_{12}N_4O_6$

USP Endotoxin RS

#### Identification—

**A: Infrared Absorption** (197K)—

**Test specimen**—To 0.5 g of Dantrolene Sodium for Injection, add 10 mL of 0.1 N hydrochloric acid and 10 mL of ethyl acetate, and mix. Allow the phases to separate, and transfer the upper ethyl acetate phase to a suitable glass container. Evaporate the solvent, dry the residue at 105° for 10 minutes, and use the residue.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 0.5 USP Endotoxin Unit per mg of dantrolene sodium.

**Sterility Tests** (71): meets the requirements.

**Uniformity of dosage units** (905): meets the requirements.

**pH** (791)—Dissolve the contents of 1 vial in 60 mL of USP Water for Injection: the pH is between 8.8 and 11.0.

**Water Determination, Method Ia** (921): not more than 3.0%.



**Related compounds—**

**Mobile phase and Diluent**—Proceed as directed in the Assay.

**Standard solution**—Transfer 10 mg of USP Dantrolene Related Compound B RS, accurately weighed, into a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume to obtain a solution having a known concentration of about 0.2 mg per mL. Dilute with *Diluent* to obtain a solution having a known concentration of about 0.002 mg per mL of dantrolene related compound B.

**Test solution**—Use the Assay preparation.

**Chromatographic system**—Proceed as directed in the Assay. Chromatograph the *Standard solution*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections for dantrolene related compound B is not more than 5.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the percentage of dantrolene related compound B in the portion of Dantrolene Sodium for Injection taken by the formula:

$$100(r_U/r_S)(C_S/C_T)$$

in which  $r_U$  is the peak response for dantrolene related compound B obtained from the *Test solution*;  $r_S$  is the corresponding peak response in the *Standard solution*;  $C_S$  is the concentration, in mg per mL, of dantrolene related compound B in the *Standard solution*; and  $C_T$  is the concentration, in mg per mL, of dantrolene sodium hydrate in the *Test solution*. Not more than 8% of dantrolene related compound B is found.

**Other requirements:** meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay—**

**Buffer**—Dissolve 3.3 g of ammonium acetate in 1 L of water, and adjust with acetic acid to a pH of  $4.5 \pm 0.1$ .

**Solution A**—Prepare a filtered and degassed mixture of *Buffer*, acetonitrile, and glacial acetic acid (120:80:7).

**Solution B**—Prepare a filtered and degassed mixture of acetonitrile and water (70:30).

**Mobile phase**—Use variable mixtures of *Solution A* and *Solution B*, as directed for *Chromatographic system*. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Diluent**—Prepare a mixture of acetonitrile and water (60:40).

**Standard preparation**—Transfer 40 mg of USP Dantrolene RS, accurately weighed, into a 50-mL volumetric flask, and dissolve in 2.5 mL of dimethylformamide. Add 2.5 mL of glacial acetic acid, and dilute with acetone to volume to obtain a solution having a known concentration of about 0.8 mg per mL. Dilute this solution with *Diluent* to obtain a solution having a known concentration of about 0.08 mg per mL of dantrolene.

**Assay preparation**—Using 70 mL of water for each vial, transfer the entire contents of the required number of vials to a suitable flask necessary to obtain a solution having a known concentration of about 0.1 mg of dantrolene sodium hydrate per mL. Sonicate for 2 to 5 minutes to dissolve the sample. Dilute with *Diluent* to volume.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 365-nm detector and a 4.6-mm  $\times$  15-cm column that contains 5- $\mu$ m packing

L1. The flow rate is about 1.5 mL per minute. The chromatograph is programmed as follows.

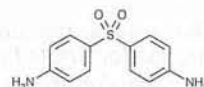
Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–8	100	0	isocratic
8–8.1	100→0	0→100	linear gradient
8.1–13	0	100	isocratic
13–13.1	0→100	100→0	linear gradient
13.1–20	100	0	re-equilibration

Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the tailing factor is not more than 1.5; and the relative standard deviation for replicate injections for dantrolene is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the peak responses for dantrolene. Calculate the percentage of  $C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$  in the portion of Dantrolene Sodium for Injection taken by the formula:

$$(399.29/314.25)(r_U/r_S)(C_S/C_U)$$

in which 399.29 and 314.25 are the molecular weights of dantrolene sodium hydrate and dantrolene, respectively;  $r_U$  and  $r_S$  are the peak responses for dantrolene obtained from the *Assay preparation* and the *Standard preparation*, respectively;  $C_S$  is the concentration, in mg per mL, of dantrolene in the *Standard preparation*; and  $C_U$  is the concentration, in mg per mL, of dantrolene sodium hydrate in the *Assay preparation*.

**Dapsone**

$C_{12}H_{12}N_2O_2S$

248.30

Benzenamine, 4,4'-sulfonylbis-;  
4,4'-Sulfonyldianiline [80-08-0].

**DEFINITION**

Dapsone contains NLT 98.0% and NMT 102.0% of dapsone ( $C_{12}H_{12}N_2O_2S$ ), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

**ASSAY**• **PROCEDURE**

**Mobile phase:** Transfer 100 mL of isopropyl alcohol, 100 mL of acetonitrile, and 100 mL of ethyl acetate to a 1000-mL volumetric flask. Add hexane to volume without mixing, then mix, and allow the mixture to cool to room temperature.

**Standard solution:** 25  $\mu$ g/mL of USP Dapsone RS in *Mobile phase*

**Sample solution:** 25  $\mu$ g/mL of Dapsone in the *Mobile phase*



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)**Mode:** LC**Detector:** UV 254 nm**Column:** 4-mm × 30-cm; 10-μm diameter packing L3**Flow rate:** 1 mL/min**Injection volume:** 10 μL**Run time:** Two times the retention time of Dapsone**System suitability****Sample:** *Standard solution***Suitability requirements****Relative standard deviation:** NMT 2%**Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of dapsone (C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S) in the portion of Dapsone taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response of the *Sample solution* $r_S$  = peak response of the *Standard solution* $C_S$  = concentration of USP Dapsone RS in the *Standard solution* (μg/mL) $C_U$  = concentration of Dapsone in the *Sample solution* (μg/mL)**Acceptance criteria:** 98.0%–102.0% on the dried basis**IMPURITIES**• **RESIDUE ON IGNITION** (281): NMT 0.1%• **SELENIUM** (291)**Sample:** 100-mg sample mixed with 100 mg of magnesium oxide**Acceptance criteria:** NMT 30 ppm• **ORGANIC IMPURITIES****Standard solution A:** 12.5 mg/mL of USP Dapsone RS in methanol**Standard solution B:** 62.5 μg/mL of USP Dapsone RS in methanol from *Standard solution A***Sample solution:** 12.5 mg/mL of Dapsone in methanol**Chromatographic system**(See *Chromatography* (621), *Thin-Layer Chromatography*.)**Mode:** TLC**Adsorbent:** 150- to 200-μm layer of chromatographic silica gel**Application volume:** 4 μL**Developing solvent system:** Acetone, chloroform, *n*-butyl alcohol, and formic acid (15:60:15:10). Prepare the solvent system fresh daily. Equilibrate the chromatographic chamber with the solvent system for 30 min prior to developing the chromatographic plate.**Spray reagent:** 0.1% (w/v) solution of 4-dimethylaminocinnamaldehyde in glacial acetic acid and water (1:1)**Analysis****Samples:** *Standard solution A* and *Standard solution B* and *Sample solution*Position the plate in a chromatographic chamber, and develop the chromatograms in the developing solvent system until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber and air-dry. Spray the plate lightly with *Spray reagent*. Examine the spots that are developed immediately, and compare the intensities of any secondary spots observed in the *Sample solution* with those of the principal spots of the *Standard solutions*.**Acceptance criteria:** No secondary spot from the chromatogram of the *Sample solution* is larger or more intense than the principal spot of *Standard solution B*(0.5%), and the sum of the intensities of all the secondary spots of the *Sample solution* corresponds to NMT 1.0%.**SPECIFIC TESTS**• **LOSS ON DRYING** (731)**Analysis:** Dry at 105° for 3 h.**Acceptance criteria:** NMT 1.5%**ADDITIONAL REQUIREMENTS**• **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.• **USP REFERENCE STANDARDS** (11)

USP Dapsone RS

**Dapsone Compounded Oral Suspension****DEFINITION**Dapsone Compounded Oral Suspension contains NLT 90.0% and NMT 110.0% of the labeled amount of dapsone (C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S).Prepare Dapsone Compounded Oral Suspension 2 mg/mL as follows (see *Pharmaceutical Compounding—Nonsterile Preparations* (795)).

Dapsone tablets <sup>a</sup> equivalent to	200 mg of dapsone
Vehicle: a 1:1 mixture of Ora-Sweet <sup>b</sup> and Ora-Plus <sup>b</sup> , a sufficient quantity to make	100 mL

<sup>a</sup> Dapsone 25-mg tablets, Jacobus Pharmaceutical Company, Princeton, NJ.<sup>b</sup> Paddock Laboratories, Minneapolis, MN.Calculate the required quantity of each ingredient for the total amount to be prepared. Place the required number of *Dapsone tablets* in a suitable mortar, and comminute to a fine powder. Add the *Vehicle* in small portions, and triturate to make a smooth paste. Add increasing volumes of the *Vehicle* to make a dapsone liquid that is pourable. Transfer the contents of the mortar, stepwise and quantitatively, to a calibrated bottle. Add enough of the *Vehicle* to bring to final volume, and mix well.**ASSAY**• **PROCEDURE****Solution A:** 50 mM ammonium phosphate adjusted to a pH of 4.6**Mobile phase:** Acetonitrile and *Solution A* (12:88). Filter and degas.**Internal standard solution:** 1.0 mg/mL of diazoxide in methanol**Standard stock solution:** 2.0 mg/mL of USP Dapsone RS in methanol**Standard solution:** Pipet 2.5 mL of *Standard stock solution* into a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 50 μg/mL of dapsone and 50 μg/mL of diazoxide. Centrifuge.**Sample solution:** Shake thoroughly by hand each bottle of Oral Suspension. Pipet 2.5 mL of Oral Suspension into a 100-mL volumetric flask, add 5.0 mL of *Internal standard solution*, and dilute with *Mobile phase* to volume to obtain a solution with a nominal concentration of 50 μg/mL of dapsone and 50 μg/mL of diazoxide. Centrifuge.



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 295 nm

Column: 3.0-mm × 15-cm; 5-μm packing L1

Column temperature: 40°

Flow rate: 0.7 mL/min

Injection volume: 10 μL

**System suitability**Sample: *Standard solution*

[NOTE—The retention times for dapsone and diazoxide are about 8.9 and 12.9 min, respectively.]

**Suitability requirements**

Relative standard deviation: NMT 2.3% for replicate injections

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of dapsone (C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S) in the portion of Oral Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

 $R_U$  = peak response ratio of dapsone to the internal standard from the *Sample solution* $R_S$  = peak response ratio of dapsone to the internal standard from the *Standard solution* $C_S$  = concentration of USP Dapsone RS in the *Standard solution* (μg/mL) $C_U$  = nominal concentration of dapsone in the *Sample solution* (μg/mL)

Acceptance criteria: 90.0%–110.0%

**SPECIFIC TESTS**

- **PH** (791): 3.8–4.8

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Package in tight, light-resistant containers. Store in a refrigerator or at controlled room temperature.
- **BEYOND-USE DATE:** NMT 90 days after the date on which it was compounded when stored in a refrigerator or at controlled room temperature
- **LABELING:** Label it to indicate that it is to be well shaken before use, and to state the *Beyond-Use Date*.
- **USP REFERENCE STANDARDS** (11)  
USP Dapsone RS

**Dapsone Tablets****DEFINITION**Dapsone Tablets contain NLT 92.5% and NMT 107.5% of the labeled amount of dapsone (C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S).**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197K)

**Sample solution:** Transfer a quantity of finely powdered Tablets, equivalent to 100 mg of dapsone, to a suitable container, add 5 mL of acetone, shake for 5 min, filter, and evaporate the filtrate to dryness. Dry this residue at 105° for 1 h.

Acceptance criteria: Meets the requirements

- **B. ULTRAVIOLET ABSORPTION** (197U)

**Sample solution:** Triturate a quantity of finely powdered Tablets, equivalent to 100 mg of dapsone, with 50 mL of methanol, and filter. Dilute a portion of the filtrate with methanol to make approximately a 1 in 200,000 solution.

Acceptance criteria: Meets the requirements

**ASSAY**• **PROCEDURE**

**Mobile phase:** Transfer 100 mL of isopropyl alcohol, 100 mL of acetonitrile, and 100 mL of ethyl acetate to a 1000-mL volumetric flask. Add hexane to volume without mixing, then mix, and allow the mixture to cool to room temperature.

**Standard solution:** 25 μg/mL of USP Dapsone RS in *Mobile phase*

**Sample solution:** Nominally 25 μg/mL of dapsone prepared as follows. Weigh and finely powder NLT 20 Tablets. Transfer a portion of the powder, nominally equivalent to 50 mg of dapsone, to a 200-mL volumetric flask. Add 150 mL of methanol, and place the flask in an ultrasonic bath at a temperature of 35° for 15 min, with occasional shaking. Allow to cool to room temperature, and add methanol to volume. Centrifuge a portion of the mixture until clear. Transfer 5.0 mL of the clear supernatant to a 50-mL volumetric flask, and dilute with *Mobile phase* to volume.

**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4-mm × 30-cm; 10-μm diameter, packing L3

Injection volume: 10 μL

**System suitability**Sample: *Standard solution* (chromatograph a sufficient number)**Suitability requirements**

Relative standard deviation: NMT 2%

**Analysis**Samples: *Standard solution* and *Sample solution*Calculate the percentage of the labeled amount of dapsone (C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 $r_U$  = peak response from the *Sample solution* $r_S$  = peak response from the *Standard solution* $C_S$  = concentration of USP Dapsone RS in the *Standard solution* (μg/mL) $C_U$  = nominal concentration of dapsone in the *Sample solution* (μg/mL)

Acceptance criteria: 92.5%–107.5%

**PERFORMANCE TESTS**

- **DISSOLUTION** (711)

Medium: Dilute hydrochloric acid (2 in 100); 1000 mL

Apparatus 1: 100 rpm

Time: 60 min

Standard solution: USP Dapsone RS of a known concentration in *Medium*

**Sample solution:** Withdraw and filter a portion of the *Sample solution*. Transfer a portion of the filtrate estimated to contain 0.2 mg of dapsone to a 25-mL volumetric flask, add 5 mL of 1 N sodium hydroxide, and dilute with water to volume.

**Instrumental conditions**

Mode: UV

Analytical wavelength: 290 nm

Tolerances: NLT 75% (Q) of the labeled amount of dapsone (C<sub>12</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS** (905)

**Procedure for content uniformity**

Standard solution: 8 μg/mL of USP Dapsone RS in methanol

**Sample solution:** Nominally 8 μg/mL of dapsone prepared as follows. To 1 Tablet in a 100-mL volumetric flask add 2.0 mL of water, and allow to stand for 30 min, swirling occasionally. Add 70 mL of methanol, and place the flask in an ultrasonic bath until the specimen is completely dispersed. Add methanol to vol-



ume, and centrifuge a portion of the mixture. Quantitatively dilute a measured volume of the clear supernatant with methanol.

#### Instrumental conditions

Mode: UV

Analytical wavelength: 296 nm

Cell: 1 cm

Blank: Methanol

#### Analysis

**Samples:** *Standard solution* and *Sample solution*.

Calculate the percentage of the labeled amount of dapsone ( $C_{12}H_{12}N_2O_2S$ ) in the Tablet taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Dapsone RS in the *Standard solution* ( $\mu\text{g/mL}$ )

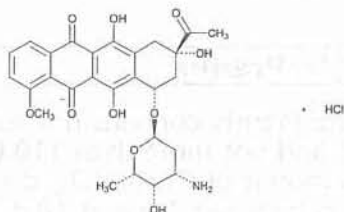
$C_U$  = nominal concentration of dapsone in the *Sample solution* ( $\mu\text{g/mL}$ )

Acceptance criteria: Meet the requirements

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed, light-resistant containers.
- **USP REFERENCE STANDARDS** (11)  
USP Dapsone RS

## Daunorubicin Hydrochloride



$C_{27}H_{29}NO_{10} \cdot HCl$  563.98

5,12-Naphthacenedione, 8-acetyl-10-[(3-amino-2,3,6-trideoxy- $\alpha$ -L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-, (8*S*-*cis*)-, hydrochloride.

(1*S*,3*S*)-3-Acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl 3-amino-2,3,6-trideoxy- $\alpha$ -L-lyxo-hexopyranoside hydrochloride [23541-50-6].

» Daunorubicin Hydrochloride has a potency equivalent to not less than 842  $\mu\text{g}$  and not more than 1030  $\mu\text{g}$  of  $C_{27}H_{29}NO_{10}$  per mg.

**Caution**—Great care should be taken to prevent inhaling particles of Daunorubicin Hydrochloride and exposing the skin to it.

**Packaging and storage**—Preserve in tight containers, protected from light and excessive heat.

**USP Reference standards** (11)—

USP Daunorubicin Hydrochloride RS

#### Identification—

**A:** The IR absorption spectrum of a potassium bromide dispersion of it exhibits maxima only at the same wavelengths as that of a similar preparation of USP Daunorubicin Hydrochloride RS.

**B:** The retention time of the main peak obtained with the *Assay preparation* corresponds to that obtained with the *Standard preparation* as directed in the *Assay*.

**Crystallinity** (695): meets the requirements.

**pH** (791): between 4.5 and 6.5, in a solution containing 5 mg per mL.

**Water Determination, Method I** (921): not more than 3.0%.

#### Assay—

**Mobile phase**—Mix 62 volumes of water and 38 volumes of acetonitrile, and adjust with phosphoric acid to a pH of  $2.2 \pm 0.2$ . The acetonitrile concentration may be varied to meet system suitability requirements and to provide a suitable elution time for daunorubicin. Filter the solution through a membrane filter (1  $\mu\text{m}$  or finer porosity), and degas.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Daunorubicin Hydrochloride RS in *Mobile phase* to obtain a solution having a known concentration of about 250  $\mu\text{g}$  of daunorubicin per mL.

**Resolution solution**—Prepare a solution of doxorubicin hydrochloride in the *Standard preparation* containing about 250  $\mu\text{g}$  per mL.

**Assay preparation**—Transfer about 25 mg of Daunorubicin Hydrochloride, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.7 for doxorubicin and 1.0 for daunorubicin; and the resolution,  $R$ , between the doxorubicin peak and the daunorubicin peak is not less than 3. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 5  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the potency, in  $\mu\text{g}$  of  $C_{27}H_{29}NO_{10}$  per mg, taken by the formula:

$$100(C/W)(r_U/r_S)$$

in which  $C$  is the concentration, in  $\mu\text{g}$  per mL, of daunorubicin in the *Standard preparation*;  $W$  is the weight, in mg, of Daunorubicin Hydrochloride taken; and  $r_U$  and  $r_S$  are the daunorubicin peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Daunorubicin Hydrochloride for Injection

» Daunorubicin Hydrochloride for Injection is a sterile mixture of Daunorubicin Hydrochloride and Mannitol. It contains the equivalent of not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{27}H_{29}NO_{10}$ .

#### Change to read:

**Packaging and storage**—Preserve as described in **•Packaging and Storage Requirements** (659), **Injection Packaging, Packaging for constitution** (CN 1-May-2017); protect from light.



**USP Reference standards** (11)—

USP Daunorubicin Hydrochloride RS  
USP Endotoxin RS

**Constituted solution**—At the time of use, it meets the requirements for *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.

**Identification**—The retention time of the main peak obtained with the *Assay preparation* corresponds to that obtained with the *Standard preparation* as directed in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 4.3 USP Endotoxin Units per mg of daunorubicin.

**pH** (791): between 4.5 and 6.5, in the solution constituted as directed in the labeling.

**Water Determination, Method I** (921): not more than 3.0%, the *Test Preparation* being prepared as directed for a hygroscopic specimen.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—

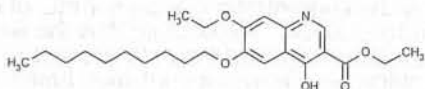
*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Daunorubicin Hydrochloride*.

*Assay preparation*—Transfer the contents of 1 vial of Daunorubicin Hydrochloride for Injection with the aid of *Mobile phase* to an appropriate volumetric flask, and dilute with *Mobile phase* to volume to obtain a solution containing about 0.25 mg of daunorubicin per mL.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Daunorubicin Hydrochloride*. Calculate the quantity, in mg, of  $C_{27}H_{29}NO_{10}$  in the vial of Daunorubicin Hydrochloride for Injection taken by the formula:

$$(CV/1000)(r_U/r_S)$$

in which *V* is the volume, in mL, of the *Assay preparation*, and the other terms are as defined therein.

**Decoquinat**

$C_{24}H_{35}NO_5$  417.54

3-Quinolinecarboxylic acid, 6-(decyloxy)-7-ethoxy-4-hydroxy-, ethyl ester.

Ethyl 6-(decyloxy)-7-ethoxy-4-hydroxy-3-quinolinecarboxylate [18507-89-6].

» Decoquinat contains not less than 99.0 percent and not more than 101.0 percent of  $C_{24}H_{35}NO_5$ , calculated on the dried basis.

**Packaging and storage**—Preserve in tight containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Decoquinat RS

**Identification**—

**A: Infrared Absorption** (197K).

**B:** Transfer about 40 mg of it, accurately weighed, to a 100-mL volumetric flask, add 10 mL of hot chloroform, swirl to dissolve, and while still warm add about 60 mL of dehydrated alcohol. Allow to cool, dilute with dehydrated alcohol to volume, and mix. Promptly transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with

dehydrated alcohol to volume, and mix. Transfer 10.0 mL of this solution to a third 100-mL volumetric flask, add 10 mL of 0.1 N hydrochloric acid, dilute with dehydrated alcohol to volume, and mix: the UV absorption spectrum of this solution exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Decoquinat RS, concurrently measured, and the respective absorptivities, calculated on the dried basis, at the wavelength of maximum absorption at about 265 nm do not differ by more than 2.5%.

**Loss on drying** (731)—Dry it at 105° to constant weight: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.1%.

**Ordinary impurities** (466)—

*Test solution:* chloroform, prepared with the aid of heat.

*Standard solution:* chloroform, using dilutions of the *Test solution*.

*Eluant:* a mixture of chloroform, dehydrated alcohol, and anhydrous formic acid (85:10:5).

*Visualization:* 1.

*Tolerances:* no impurity exceeds 1%, and the total does not exceed 2%.

**Assay**—Dissolve about 1000 mg of Decoquinat, accurately weighed, in 100 mL of glacial acetic acid, with the aid of gentle heat. Allow to cool, add 1 drop of crystal violet TS, and titrate with 0.1 N perchloric acid VS to a green endpoint. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 41.76 mg of  $C_{24}H_{35}NO_5$ .

**Decoquinat Premix**

» Decoquinat Premix contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{24}H_{35}NO_5$ , the labeled amount being between 1 g and 10 g per 100 g of Premix.

**Packaging and storage**—Preserve in well-closed containers.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Decoquinat RS

**Identification**—To an accurately weighed quantity of it, equivalent to about 100 mg of decoquinat, add 40 mL of chloroform, and heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Use the filtrate as the test solution. Apply 10-μL portions of the test solution and of a Standard solution in chloroform containing 2.5 mg of USP Decoquinat RS per mL to the starting line of a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and alcohol (70:30) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, and allow to dry in a current of air. Locate the spots under short-wavelength UV light: the *R<sub>f</sub>* value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

**Assay**—Extract an accurately weighed quantity of Premix, equivalent to about 200 mg of decoquinat, with 50 mL of chloroform in a small continuous extraction apparatus for 8 reflux cycles. Transfer the extract to a 100-mL volumetric

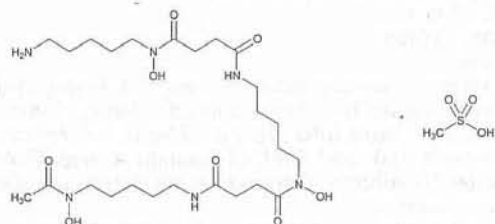


flask with the aid of chloroform, cool, dilute with chloroform to volume, and mix. Transfer 5.0 mL of this solution to a second 100-mL volumetric flask, dilute with dehydrated alcohol to volume, and mix. Transfer 5.0 mL of this solution to a third 100-mL volumetric flask, add 10 mL of 0.1 N hydrochloric acid, dilute with dehydrated alcohol to volume, and mix (*Assay preparation*). Transfer 50 mg of USP Decoquinat RS, accurately weighed, to a 100-mL volumetric flask, add 10 mL of hot chloroform, swirl to dissolve, and while still warm slowly add 70 mL of dehydrated alcohol. Allow to cool, dilute with dehydrated alcohol to volume, and mix. Immediately transfer 10.0 mL of this solution to a second 100-mL volumetric flask, dilute with dehydrated alcohol to volume, and mix. Transfer 10.0 mL of this solution to a third 100-mL volumetric flask, add 10 mL of 0.1 N hydrochloric acid, dilute with dehydrated alcohol to volume, and mix. This *Standard preparation* contains about 0.005 mg of USP Decoquinat RS per mL. Concomitantly determine the absorbances of the *Standard preparation* and the *Assay preparation* at the wavelength of maximum absorbance at about 265 nm, with a spectrophotometer, using a mixture of dehydrated alcohol, 0.1 N hydrochloric acid, and chloroform (90:10:0.25) as the blank. Calculate the percentage of  $C_{24}H_{35}NO_5$  in the portion of Premix taken by the formula:

$$4000(C/W)(A_U/A_S)$$

in which C is the concentration, in mg per mL, of USP Decoquinat RS in the *Standard preparation*; W is the quantity, in g, of Premix taken to prepare the *Assay preparation*; and  $A_U$  and  $A_S$  are the absorbances of the *Assay preparation* and the *Standard preparation*, respectively.

## Deferoxamine Mesylate



$C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$  656.79  
Butanediamide, N'-[5-[[4-[[5-(acetylhydroxyamino)pentyl]-amino]-1,4-dioxobutyl]hydroxyamino]pentyl]-N-(5-aminopentyl)-N-hydroxy-, monomethanesulfonate;  
N-[5-[3-[(5-Aminopentyl)hydroxycarbonyl]propionamido]pentyl]-3-[[5-(N-hydroxyacetamido)pentyl]carbonyl]propionohydroxamic acid monomethanesulfonate (salt) [138-14-7].

### DEFINITION

Deferoxamine Mesylate contains NLT 93.0% and NMT 102.0% of deferoxamine mesylate ( $C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$ ), calculated on the anhydrous basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Solution A:** 1.32 g/L of dibasic ammonium phosphate in water. Adjust with phosphoric acid to a pH of 3.0.

**Solution B:** Acetonitrile and *Solution A* (1:1)  
**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	88	12
20	80	20
35	57.5	42.5
35.1	88	12
40	88	12

**Diluent:** Acetonitrile and water (6:94)

**Standard solution:** 1.0 mg/mL of USP Deferoxamine Mesylate RS in *Diluent*

**Sample solution:** 1.0 mg/mL of Deferoxamine Mesylate in *Diluent*

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 7.5-cm; 3.5-μm packing L1

**Temperatures**

**Column:** 32°

**Autosampler:** 5°

**Flow rate:** 1.5 mL/min

**Injection volume:** 20 μL

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Relative standard deviation:** NMT 0.73%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of deferoxamine mesylate ( $C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$ ) in the portion of Deferoxamine Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of deferoxamine from the *Sample solution*

$r_S$  = peak response of deferoxamine from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** 93.0%–102.0% on the anhydrous basis

### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.1%, 2.0 g being used
- **CHLORIDE AND SULFATE, Chloride** (221): NMT 0.012%; a 1.2-g portion shows no more chloride than corresponds to 0.20 mL of 0.020 N hydrochloric acid.
- **CHLORIDE AND SULFATE, Sulfate** (221): NMT 0.04%; a 0.5-g portion shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid.

### Delete the following:

- **HEAVY METALS, Method II** (231): NMT 10 ppm (Official 1-Jan-2018)

### ORGANIC IMPURITIES

**Solution A, Solution B, Diluent, Mobile phase, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard stock solution:** Use the *Standard solution*, prepared as directed in the *Assay*. [NOTE—USP Deferoxamine Mesylate RS contains impurity A as a minor component.]

**Standard solution:** 0.01 mg/mL of USP Deferoxamine Mesylate RS in *Diluent* from the *Standard stock solution*



**System suitability**

**Samples:** *Standard stock solution* and *Standard solution*  
**Suitability requirements**

**Resolution:** NLT 2.0 between the impurity A and deferoxamine peaks, *Standard stock solution*

**Relative standard deviation:** NMT 5.0% for the deferoxamine peak, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*  
 Calculate the percentage of each impurity in the portion of Deferoxamine Mesylate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of deferoxamine from the *Standard solution*

$C_S$  = concentration of the *Standard solution* (mg/mL)

$C_U$  = concentration of the *Sample solution* (mg/mL)

**Acceptance criteria:** See Table 2.

[NOTE—The reporting level for impurities is 0.04%.]

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Impurity A <sup>a,b</sup>	0.85–0.87	3.0 <sup>c</sup>
Deferoxamine	1.0	—
Any unspecified impurity	—	1.0
Total impurities eluting before deferoxamine	—	5.0
Total impurities eluting after deferoxamine	—	2.0

<sup>a</sup> Des-methylene impurity (desferrioxamine A<sub>1</sub> and/or other desferrioxamines).

<sup>b</sup> All des-methylene impurities that elute in the 0.85–0.87 range should be treated as a single impurity. Where the cluster of unresolved peaks in this range is present, it should be integrated together as one peak to determine compliance.

<sup>c</sup> The acceptance criterion of NMT 3.0% applies to the sum of the peaks in the specified range.

**SPECIFIC TESTS**

- **PH (791):** 4.0–6.0, in a solution (1 in 100)
- **WATER DETERMINATION, Method I (921):** NMT 2.0%
- **STERILITY TESTS (71):** Where the label states that Deferoxamine Mesylate is sterile, it meets the requirements.
- **BACTERIAL ENDOTOXINS TEST (85):** Where the label states that Deferoxamine Mesylate is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.33 USP Endotoxin Unit/mg of deferoxamine mesylate.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at room temperature.
- **LABELING:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

- **USP REFERENCE STANDARDS (11)**  
 USP Deferoxamine Mesylate RS  
 USP Endotoxin RS

**Deferoxamine Mesylate for Injection****DEFINITION**

Deferoxamine Mesylate for Injection contains NLT 90.0% and NMT 110.0% of the labeled amount of deferoxamine mesylate ( $C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$ ).

**IDENTIFICATION**• **A.**

**Sample:** 5 mg of Deferoxamine Mesylate for Injection

**Analysis:** Dissolve the *Sample* in 5 mL of water, add 2 mL of tribasic sodium phosphate solution (1 in 200), mix, then add 10 drops of  $\beta$ -naphthoquinone-4-sodium sulfonate solution (1 in 40).

**Acceptance criteria:** A blackish brown color is produced.

**ASSAY**• **PROCEDURE**

**Solution A:** Dissolve 6.7 g of ferric chloride in dilute hydrochloric acid (1 in 100) in a 100-mL volumetric flask. Add dilute hydrochloric acid (1 in 100) to volume.  
**Standard solution:** 1 mg/mL of USP Deferoxamine Mesylate RS

**Sample solution:** Nominally 1 mg/mL, prepared as follows. Constitute the contents of 1 vial in water, and dilute with water to volume.

**Instrumental conditions**

(See *Ultraviolet-Visible Spectroscopy* (857).)

**Mode:** Vis

**Analytical wavelength:** 485 nm

**Cell:** 1 cm

**Blank:** Water

**Analysis**

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
 Pipet 2 mL each of the *Standard solution*, *Sample solution*, and *Blank* into separate 25-mL volumetric flasks. To each flask add 3 mL of *Solution A*, and dilute with water to volume. Concomitantly determine the absorbances.

Calculate the percentage of the labeled amount of deferoxamine mesylate ( $C_{25}H_{48}N_6O_8 \cdot CH_4O_3S$ ) in the portion of Deferoxamine Mesylate for Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Deferoxamine Mesylate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of deferoxamine mesylate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**PERFORMANCE TESTS**

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements

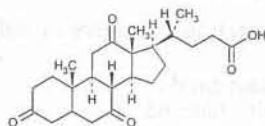
**SPECIFIC TESTS**

- **PH (791):** 4.0–6.0, in a solution (1 in 100)
- **WATER DETERMINATION, Method I (921):** NMT 1.5%
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 0.33 USP Endotoxin Unit/mg of deferoxamine mesylate
- **CONSTITUTED SOLUTION:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* (1), *Specific Tests*, *Completeness and clarity of solutions*.



**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass.
- **USP REFERENCE STANDARDS (11)**  
USP Deferoxamine Mesylate RS  
USP Endotoxin RS

**Dehydrocholic Acid**

$C_{24}H_{34}O_5$  402.52  
Cholan-24-oic acid, 3,7,12-trioxo-, (5 $\beta$ )-;  
3,7,12-Trioxo-5 $\beta$ -cholan-24-oic acid [81-23-2].

**DEFINITION**

Dehydrocholic Acid contains NLT 98.5% and NMT 101.0% of  $C_{24}H_{34}O_5$ , calculated on the dried basis. Dehydrocholic Acid for parenteral use melts between 237° and 242°.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**

**ASSAY****• PROCEDURE**

**Sample solution:** Transfer 500 mg of Dehydrocholic Acid to a 300-mL conical flask, add 60 mL of neutralized alcohol, and warm on a steam bath until dissolved. Allow to cool.

**Analysis:** Add phenolphthalein TS and 20 mL of water to the *Sample solution*. Titrate with 0.1 N sodium hydroxide VS, adding 100 mL of water shortly before the endpoint is reached. Each mL of 0.1 N sodium hydroxide is equivalent to 40.25 mg of  $C_{24}H_{34}O_5$ .

**Acceptance criteria:** 98.5%–101.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 0.3%
- **BARIUM**

**Sample:** 2 g

**Analysis:** Boil the *Sample* with 100 mL of water for 2 min. Add 2 mL of hydrochloric acid, and again boil for 2 min. Cool, filter, and wash the filter with water until the filtrate measures 100 mL. To 10 mL of the filtrate add 1 mL of 2 N sulfuric acid.

**Acceptance criteria:** No turbidity is produced.

**Delete the following:**

- **HEAVY METALS, Method II (231):** 20 ppm (Official 1-Jan-2018)

**SPECIFIC TESTS**

- **MELTING RANGE OR TEMPERATURE (741):** 231°–242°, but the range between beginning and end of melting does not exceed 3°.
- **OPTICAL ROTATION, Specific Rotation (781S):** +29.0° to +32.5°  
**Sample solution:** 20 mg/mL in dioxane
- **LOSS ON DRYING (731):** Dry at 105° for 2 h; it loses NMT 1.0% of its weight.
- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** It meets the requirements of the test for absence of *Salmonella* species.
- **ODOR ON BOILING:** Boil 2 g with 100 mL of water for 2 min; the mixture is odorless.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
USP Dehydrocholic Acid RS

**Dehydrocholic Acid Tablets****DEFINITION**

Dehydrocholic Acid Tablets contain NLT 94.0% and NMT 106.0% of the labeled amount of  $C_{24}H_{34}O_5$ .

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**

**Sample:** Mix a quantity of finely powdered Tablets equivalent to 500 mg of dehydrocholic acid with 15 mL of water, and add slowly, with stirring, 2 mL of sodium carbonate TS. Filter, and add to the filtrate 3 N hydrochloric acid (about 2 mL) dropwise until no more precipitate is formed. Filter the precipitate, wash with small portions of cold water until free from chloride, and dry at 105° for 2 h. [NOTE—Reserve a portion of the material obtained for use in *Identification* test B.]

- **B. MELTING RANGE OR TEMPERATURE (741):** 231°–242°, but the range between beginning and end of melting does not exceed 3°.

**Sample:** Use the material reserved from *Identification* test A.

**ASSAY****• PROCEDURE**

**Sample:** Finely powder NLT 20 Tablets. Transfer a portion of the powder equivalent to 500 mg of dehydrocholic acid to a 300-mL conical flask. Add 60 mL of neutralized alcohol, and warm on a steam bath for 10 min. Allow to cool.

**Analysis:** Add phenolphthalein TS and 20 mL of water. Titrate with 0.1 N sodium hydroxide VS, adding 100 mL of water shortly before the endpoint is reached. Each mL of 0.1 N sodium hydroxide is equivalent to 40.25 mg of  $C_{24}H_{34}O_5$ .

**Acceptance criteria:** 94.0%–106.0%

**PERFORMANCE TESTS**

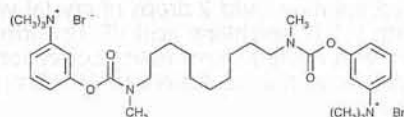
- **DISINTEGRATION (701)**  
**Time:** NMT 30 min
- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** Meets the requirements of the test for absence of *Salmonella* species

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS (11)**  
USP Dehydrocholic Acid RS

**Demecarium Bromide**

$C_{32}H_{52}Br_2N_4O_4$  716.59



Benzenaminium, 3,3'-[1,10-decanediylbis[(methylimino)carbonyloxy]]bis[N,N,N-trimethyl]-, dibromide.

(*m*-Hydroxyphenyl)trimethylammonium bromide decamethylenebis[methylcarbamate] (2:1) [56-94-0].

» Demecarium Bromide contains not less than 95.0 percent and not more than 100.5 percent of  $C_{32}H_{52}Br_2N_4O_4$ , calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Demecarium Bromide RS

#### Identification—

A: *Infrared Absorption* (197K).

B: Dissolve about 100 mg in 50 mL of 1 N sodium hydroxide, and reflux for 15 minutes. Cool, and add 3 mL of the refluxed solution to 25 mL of saturated sodium bicarbonate solution. Add, with mixing, 4 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride solution (1.5 in 10,000) and 2 mL of sodium hypochlorite solution (1.5 in 20,000); a violet-blue color is produced.

C: Dissolve about 50 mg in 20 mL of water, add 10 mL of a 1 in 50 solution of ammonium reineckate in methanol, and allow to stand for 30 minutes with occasional swirling; a pink reineckate of demecarium forms, and it melts between 131° and 136°, with decomposition.

D: A solution of it responds to the tests for *Bromide* (191).

**pH** (791): between 5.0 and 7.0, in a solution (1 in 100).

**Water Determination**, *Method I* (921): not more than 2.0%.

**Residue on ignition** (281): not more than 0.1%.

#### Delete the following:

• **Heavy metals**, *Method I* (231): 0.002%. • (Official 1-Jan-2018)

#### Limit of *m*-trimethylammoniofenol bromide—

**Control solution**—Dissolve 100 mg of *m*-dimethylaminophenol in 10 mL of alcohol in a 1000-mL volumetric flask, dilute with water to volume, and mix. Pipet 1 mL of this solution into a 500-mL volumetric flask, dilute with water to volume, and mix.

**Test solution**—Transfer 100 mg of Demecarium Bromide to a 100-mL volumetric flask, add water to volume, and mix.

**Procedure**—Pipet 25 mL of the *Test solution* into a glass-stoppered, 50-mL centrifuge tube, and pipet 25 mL of the *Control solution* into a second, similar tube. To each tube add 3 mL of pH 7.0 phosphate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*), 1 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride solution (1.5 in 10,000), 5 mL of isobutyl alcohol, and 1 mL of sodium hypochlorite solution (1.5 in 20,000). Insert the stoppers in the tubes, shake the mixtures for 5 minutes, and centrifuge; any blue color produced in the upper layer obtained from the *Test solution* is not more intense than that obtained from the *Control solution*.

**Assay**—Dissolve about 0.8 g of Demecarium Bromide, accurately weighed, in a mixture of 75 mL of glacial acetic acid and 15 mL of mercuric acetate TS, warming slightly, if necessary, to effect solution. Add 2 drops of crystal violet TS, and titrate with 0.1 N perchloric acid VS. Perform a blank determination, and make any necessary correction. Each mL of 0.1 N perchloric acid is equivalent to 35.83 mg of  $C_{32}H_{52}Br_2N_4O_4$ .

## Demecarium Bromide Ophthalmic Solution

» Demecarium Bromide Ophthalmic Solution is a sterile, aqueous solution of Demecarium Bromide. It contains not less than 92.0 percent and not more than 108.0 percent of the labeled amount of  $C_{32}H_{52}Br_2N_4O_4$ . It contains a suitable antimicrobial agent.

**Packaging and storage**—Preserve in tight, light-resistant containers.

**USP Reference standards** (11)—

USP Demecarium Bromide RS

#### Identification—

A: Mix about 10 mL with 12.5 mL of 1 N sodium hydroxide, and proceed as directed in *Identification* test B under *Demecarium Bromide*, beginning with "reflux for 15 minutes": a violet-blue color is produced.

B: To 10 mL add 5 mL of a 1 in 50 solution of ammonium reineckate in methanol, and allow to stand for 30 minutes with occasional swirling; a pink reineckate of demecarium forms, and it melts between 131° and 136°, with decomposition.

C: It responds to the tests for *Bromide* (191).

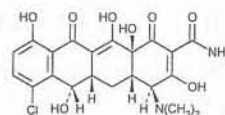
**Sterility Tests** (71): meets the requirements.

**Assay**—Into each of two 50-mL volumetric flasks marked 1 and 2 pipet a volume of Ophthalmic Solution, equivalent to about 2.5 mg of demecarium bromide. Into each of two additional 50-mL volumetric flasks, marked 3 and 4, pipet 5.0 mL of a freshly prepared Standard solution made by dissolving about 50 mg of USP Demecarium Bromide RS, accurately weighed, in 100.0 mL of water. To flasks 1 and 3, add 1 N sodium hydroxide to volume, and mix. Transfer 10.0 mL of each of these solutions to separate glass-stoppered tubes, insert the stoppers loosely, heat the tubes in a water bath for 15 minutes, then cool to room temperature. The concentration of USP Demecarium Bromide RS in the Standard solution is about 50 µg per mL. To flasks 2 and 4, add pH 10.0 alkaline borate buffer (see under *Solutions* in the section *Reagents, Indicators, and Solutions*) to volume, and mix. Concomitantly determine the absorbances of the two sodium hydroxide solutions at the wavelength of maximum absorbance at about 292 nm, with a suitable spectrophotometer, using the corresponding borate buffer solutions as the respective solvent blanks. Calculate the quantity, in mg, of  $C_{32}H_{52}Br_2N_4O_4$  in each mL of the Ophthalmic Solution taken by the formula:

$$(50C / V)(A_U / A_S)$$

in which C is the concentration, in mg per mL, of USP Demecarium Bromide RS in the Standard solution; V is the volume, in mL, of Ophthalmic Solution taken; and  $A_U$  and  $A_S$  are the absorbances of the sodium hydroxide solution from the Ophthalmic Solution and the Standard solution, respectively.

## Demeclocycline



$C_{21}H_{21}ClN_2O_8$  464.85



2-Naphthacene-carboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-, [4S-(4 $\alpha$ ,4a $\alpha$ ,5a $\alpha$ ,6 $\beta$ ,12a $\alpha$ )]-. 7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-2-naphthacene-carboxamide [127-33-3].  
Sesquihydrate 491.88 [13215-10-6].

» Demeclocycline has a potency equivalent to not less than 970  $\mu\text{g}$  of demeclocycline hydrochloride ( $\text{C}_{21}\text{H}_{21}\text{ClN}_2\text{O}_8 \cdot \text{HCl}$ ) per mg, calculated on the anhydrous basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Demeclocycline Hydrochloride RS

#### Identification—

**A:** Transfer about 40 mg, accurately weighed, to a 250-mL volumetric flask, add 2 mL of 0.1 N hydrochloric acid to dissolve, dilute with water to volume, and mix. Transfer 10.0 mL of this solution to a 100-mL volumetric flask, add about 75 mL of water and 5.0 mL of 5 N sodium hydroxide, dilute with water to volume, and mix: the UV absorption spectrum of this solution, measured at 6 minutes, accurately timed, after the addition of the sodium hydroxide, exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Demeclocycline Hydrochloride RS, concomitantly measured, and the absorptivity, calculated on the anhydrous basis, at the wavelength of maximum absorbance at about 380 nm is between 103.5% and 111.3% of that of the USP Demeclocycline Hydrochloride RS, the potency of the Reference Standard being taken into account.

**B:** Transfer 40 mg, accurately weighed, to a 200-mL volumetric flask, add 100 mL of 0.1 N hydrochloric acid, shake to dissolve, dilute with 0.1 N hydrochloric acid to volume, and mix. Transfer 5.0 mL of this solution into each of two 50-mL volumetric flasks (*Solutions 1 and 2*). Prepare similar solutions of USP Demeclocycline Hydrochloride RS (*Solutions 3 and 4*). To *Solutions 1 and 3*, add 10 mL of 6 N hydrochloric acid, and to *Solutions 2 and 4*, add 10 mL of 3 N hydrochloric acid. Heat the four flasks in a water bath for 20 minutes, cool, dilute the contents with water to volume, and mix. Determine the absorbances of *Solutions 1 and 3* at the wavelength of maximum absorbance at about 430 nm, with a suitable spectrophotometer, using *Solutions 2 and 4*, respectively, as the blanks. Determine the absorbances of *Solutions 2 and 4* at the wavelength of maximum absorbance at about 368 nm, using *Solutions 1 and 3*, respectively, as the blanks. Calculate the ratio:

$$(W_S P / 1000)(A_{368} + A_{430})_U / W_U(A_{368} + A_{430})_S$$

in which  $W_S$  is the weight, in mg, of USP Demeclocycline Hydrochloride RS taken, calculated on the dried basis;  $P$  is the potency, in  $\mu\text{g}$  per mg, of the USP Demeclocycline Hydrochloride RS;  $W_U$  is the weight, in mg, of specimen taken, calculated on the anhydrous basis; and the final two parenthetic expressions are the absorbances of the four solutions at the wavelengths indicated by the subscripts for the specimen ( $U$ ) and the Standard ( $S$ ): the ratio is between 0.97 and 1.17.

**Crystallinity** (695): meets the requirements.

**pH** (791): between 4.0 and 5.5, in a solution containing 10 mg per mL.

**Water Determination, Method I** (921): between 4.3% and 6.7%.

#### Assay—

**Mobile phase**—Transfer 80 g of tertiary butyl alcohol to a 1000-mL volumetric flask with the aid of 200 mL of water, add 100 mL of 0.2 M pH 9.0 phosphate buffer (prepared by

mixing appropriate volumes of 0.2 M dibasic potassium phosphate and 0.2 M monobasic potassium phosphate), 150 mL of 0.02 M tetrabutylammonium hydrogen sulfate (adjusted with sodium hydroxide TS to a pH of 9.0), and 100 mL of 0.01 M edetate disodium (adjusted with sodium hydroxide TS to a pH of 9.0). Dilute with water to volume, mix, and degas. Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)). Decreasing the amount of tertiary butyl alcohol increases the resolution.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Demeclocycline Hydrochloride RS in 0.01 N hydrochloric acid to obtain a solution having a known concentration of about 1 mg per mL.

**Assay preparation**—Transfer about 45 mg of Demeclocycline, accurately weighed, to a 50-mL volumetric flask, dissolve in 0.01 N hydrochloric acid, dilute with 0.01 N hydrochloric acid to volume, and mix.

**Resolution solution**—Prepare a solution of USP Demeclocycline Hydrochloride RS in 0.01 N hydrochloric acid, and allow to stand for 3 hours.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column containing packing L21 and maintained at  $60 \pm 0.5^\circ$ . The flow rate is about 1 mL per minute. Chromatograph the *Resolution solution*, and record the responses as directed for *Procedure*: the relative retention times are about 0.7 for epidemethylchlorotetracycline and 1.0 for demeclocycline; and the resolution between the epidemethylchlorotetracycline peak and the demeclocycline peak is not less than 3.0. Chromatograph the *Standard preparation*, and record the responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—[NOTE—Use peak areas where peak responses are indicated.] Separately inject equal volumes (about 20  $\mu\text{L}$ ) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in  $\mu\text{g}$ , of demeclocycline hydrochloride ( $\text{C}_{21}\text{H}_{21}\text{ClN}_2\text{O}_8 \cdot \text{HCl}$ ) equivalent in each mg of the Demeclocycline taken by the formula:

$$50(CE / W)(r_U / r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Demeclocycline Hydrochloride RS in the *Standard preparation*;  $E$  is the demeclocycline hydrochloride equivalent, in  $\mu\text{g}$  per mg, of USP Demeclocycline Hydrochloride RS;  $W$  is the quantity, in mg, of the Demeclocycline taken; and  $r_U$  and  $r_S$  are the demeclocycline peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Demeclocycline Oral Suspension

» Demeclocycline Oral Suspension contains the equivalent of not less than 90.0 percent and not more than 125.0 percent of the labeled amount of demeclocycline hydrochloride ( $\text{C}_{21}\text{H}_{21}\text{ClN}_2\text{O}_8 \cdot \text{HCl}$ ). It may contain one or more suitable buffers, preservatives, stabilizers, and suspending agents.

**Packaging and storage**—Preserve in tight containers, protected from light.

#### USP Reference standards (11)—

USP Demeclocycline Hydrochloride RS

**Identification**—To an accurately measured volume of Oral Suspension, equivalent to about 50 mg of demeclocycline



hydrochloride, add 50 mL of methanol, shake, and allow the mixture to settle. Using the clear supernatant as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

#### Uniformity of dosage units (905)—

FOR ORAL SUSPENSION PACKAGED IN SINGLE-UNIT CONTAINERS: meets the requirements.

#### Deliverable volume (698)—

FOR ORAL SUSPENSION PACKAGED IN MULTIPLE-UNIT CONTAINERS: meets the requirements.

**pH** (791): between 4.0 and 5.8.

#### Assay—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Demeclocycline*.

*Assay preparation*—Transfer an accurately weighed quantity of Oral Suspension, freshly mixed and free from air bubbles, equivalent to about 50 mg of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ), to a 50-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. Sonicate for 5 minutes, and centrifuge for 5 minutes. Pass a portion of the supernatant through a suitable filter having a 1.5- $\mu$ m or finer porosity, and use the clear filtrate as the *Assay preparation*.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Demeclocycline*. Calculate the quantity, in mg, of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ) equivalent in each mL of Oral Suspension taken by the formula:

$$0.05(CE / V)(r_U / r_S)$$

in which *V* is the volume, in mL, of Oral Suspension taken; and the other terms are as defined therein.

## Demeclocycline Hydrochloride

$C_{21}H_{21}ClN_2O_8 \cdot HCl$  501.31

2-Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-, monohydrochloride, 4S-(4 $\alpha$ , 4a $\alpha$ , 5a $\alpha$ , 6 $\beta$ , 12a $\alpha$ )-  
7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride [64-73-3].

» Demeclocycline Hydrochloride has a potency of not less than 900  $\mu$ g of  $C_{21}H_{21}ClN_2O_8 \cdot HCl$  per mg, calculated on the dried basis.

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Demeclocycline Hydrochloride RS

#### Identification—

**A:** It responds to *Identification test A* under *Demeclocycline*, except that its absorptivity, calculated on the dried basis, is between 95.8% and 104.2% of that of the USP Demeclocycline Hydrochloride RS, the potency of the Reference Standard being taken into account.

**B:** It responds to *Identification test B* under *Demeclocycline*, except that the ratio is 0.9 to 1.1.

**Crystallinity** (695): meets the requirements.

**pH** (791): between 2.0 and 3.0, in a solution containing 10 mg per mL.

**Loss on drying** (731)—Dry about 100 mg, accurately weighed, in a capillary-stoppered bottle in vacuum at a

pressure not exceeding 5 mm of mercury at 60° for 3 hours: it loses not more than 2.0% of its weight.

#### Assay—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Demeclocycline*.

*Assay preparation*—Transfer about 50 mg of Demeclocycline Hydrochloride, accurately weighed, to a 50-mL volumetric flask, dissolve in 0.01 N hydrochloric acid, dilute with 0.01 N hydrochloric acid to volume, and mix.

*Procedure*—Proceed as directed for *Procedure* in the *Assay* under *Demeclocycline*. Calculate the quantity, in  $\mu$ g, of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ) in each mg of the Demeclocycline Hydrochloride taken by the formula:

$$50(CE / W)(r_U / r_S)$$

in which *W* is the quantity, in mg, of the Demeclocycline Hydrochloride taken, and the other terms are as defined therein.

## Demeclocycline Hydrochloride Capsules

» Demeclocycline Hydrochloride Capsules contain not less than 90.0 percent and not more than 125.0 percent of the labeled amount of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ).

**Packaging and storage**—Preserve in tight, light-resistant containers.

#### USP Reference standards (11)—

USP Demeclocycline Hydrochloride RS

**Identification**—Shake a suitable quantity of Capsule contents with methanol to obtain a solution containing about 1 mg of demeclocycline hydrochloride per mL, and filter. Using the filtrate as the *Test Solution*, proceed as directed for *Method II* under *Identification—Tetracyclines* (193).

#### Dissolution (711)—

*Medium:* water; 900 mL.

*Apparatus 2:* 75 rpm.

*Time:* 45 minutes.

*Procedure*—Determine the amount of  $C_{21}H_{21}ClN_2O_8$  dissolved from UV absorption at the wavelength of maximum absorbance at about 274 nm on filtered portions of the solution under test, suitably diluted with *Dissolution Medium*, if necessary, in comparison with a Standard solution having a known concentration of USP Demeclocycline Hydrochloride RS in the same *Medium*.

**Tolerances**—Not less than 75% (Q) of the labeled amount of demeclocycline ( $C_{21}H_{21}ClN_2O_8$ ) is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Loss on drying** (731)—Dry about 100 mg of Capsule contents, accurately weighed, in a capillary-stoppered bottle in vacuum at 60° for 3 hours: the material loses not more than 2.0% of its weight, except that if the Capsules contain starch the material loses not more than 8.0% of its weight.

#### Assay—

*Mobile phase, Standard preparation, Resolution solution, and Chromatographic system*—Prepare as directed in the *Assay* under *Demeclocycline*.

*Assay preparation*—Remove, as completely as possible, the contents of not fewer than 10 Capsules, and weigh. Mix, and transfer an accurately weighed portion of the powder, equivalent to about 50 mg of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ), to a 50-mL volumetric flask,



dilute with 0.01 N hydrochloric acid to volume, and mix. Sonicate for 5 minutes, and centrifuge for 5 minutes. Pass a portion of the supernatant through a suitable filter having a 1.5- $\mu$ m or finer porosity, and use the clear filtrate as the Assay preparation.

**Procedure**—Proceed as directed for Procedure in the Assay under Demeclocycline. Calculate the quantity, in mg, of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ) in the portion of Capsule contents taken by the formula:

$$0.05CE(r_u / r_s)$$

in which the terms are as defined therein.

## Demeclocycline Hydrochloride Tablets

### DEFINITION

Demeclocycline Hydrochloride Tablets contain NLT 90.0% and NMT 125.0% of the labeled amount of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ).

### IDENTIFICATION

#### Delete the following:

#### A. IDENTIFICATION—TETRACYCLINES, Method II (193)

Sample solution: 1 mg/mL of demeclocycline hydrochloride, from finely powdered Tablets in methanol. Use the filtrate.  $\Delta_{USP40}$

#### Add the following:

- A. The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.  $\Delta_{USP40}$

### ASSAY

#### Change to read:

#### PROCEDURE

**Solution A:** Mix appropriate volumes of 0.2 M dibasic potassium phosphate and 0.2 M monobasic potassium phosphate to prepare a buffer with a pH of 9.0.

**Solution B:** 0.02 M tetrabutylammonium hydrogen sulfate. Adjust with sodium hydroxide TS to a pH of 9.0.

**Solution C:** 0.01 M edetate disodium. Adjust with sodium hydroxide TS to a pH of 9.0.

**Mobile phase:** Transfer 80 g of tertiary butyl alcohol to a 1000-mL volumetric flask with the aid of 200 mL of water. Add 100 mL of Solution A, 150 mL of Solution B, and 100 mL of Solution C. Dilute with water to volume, and degas.  $\Delta_{USP40}$

**System suitability solution:** USP Demeclocycline Hydrochloride RS in 0.01 N hydrochloric acid. Allow to stand for 3 h.

**Standard solution:** 1 mg/mL of USP Demeclocycline Hydrochloride RS in 0.01 N hydrochloric acid

**Sample solution:** Nominally 1 mg/mL of demeclocycline hydrochloride in 0.01 N hydrochloric acid. Prepare as follows. Weigh and finely powder NLT 10 Tablets. Transfer a portion, containing nominally 50 mg of demeclocycline hydrochloride, to a 50-mL volumetric flask, and dilute with 0.01 N hydrochloric acid to volume. Sonicate for 5 min, and centrifuge for 5 min. Pass a portion of the supernatant through a suitable filter of 1.5- $\mu$ m or finer pore size.

### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm  $\times$  25-cm;  $\Delta$ 8- $\mu$ m  $\Delta_{USP40}$  packing L21

Column temperature: 60  $\pm$  0.5°

Flow rate: 1 mL/min

Injection volume: 20  $\mu$ L

### System suitability

**Samples:** System suitability solution and Standard solution

[NOTE—The relative retention times for epidemethylchlorotetracycline and demeclocycline are about 0.7 and 1.0, respectively.  $\Delta_{USP40}$ ]

### Suitability requirements

**Resolution:** NLT 3.0 between the epidemethylchlorotetracycline and demeclocycline peaks, System suitability solution

**Relative standard deviation:** NMT 2.0%, Standard solution

### Analysis

**Samples:** Standard solution and Sample solution

Calculate the percentage of the labeled amount of demeclocycline hydrochloride ( $C_{21}H_{21}ClN_2O_8 \cdot HCl$ ) in the portion of Tablets taken:

$$\text{Result} = (r_u / r_s) \times (C_s / C_u) \times P \times F \times 100$$

$r_u$  = peak  $\Delta$ response  $\Delta_{USP40}$  from the Sample solution

$r_s$  = peak  $\Delta$ response  $\Delta_{USP40}$  from the Standard solution

$C_s$  = concentration of USP Demeclocycline Hydrochloride RS in the Standard solution (mg/mL)

$C_u$  = nominal concentration of demeclocycline hydrochloride in the Sample solution (mg/mL)

$P$  = potency of demeclocycline in USP Demeclocycline Hydrochloride RS ( $\mu$ g/mg)

$F$  = conversion factor, 0.001 mg/ $\mu$ g

Acceptance criteria: 90.0%–125.0%

### PERFORMANCE TESTS

#### Change to read:

#### DISSOLUTION (711)

Medium: Water; 900 mL

Apparatus 2: 75 rpm

Time: 45 min

**Standard solution:** Prepare a solution with a known concentration of USP Demeclocycline Hydrochloride RS in Medium.

**Sample solution:** Sample per Dissolution (711). A filtered portion of the solution under test suitably diluted with Medium

### Instrumental conditions

(See Ultraviolet-Visible Spectroscopy (857).)

Mode: UV

Analytical wavelength: 274 nm

### Analysis

**Samples:** Standard solution and Sample solution

$\Delta$ Calculate the percentage of the labeled amount of demeclocycline ( $C_{21}H_{21}ClN_2O_8$ ) dissolved:

$$\text{Result} = (A_u / A_s) \times (C_s / L) \times D \times V \times P \times F \times 100$$

$A_u$  = absorbance of the Sample solution

$A_s$  = absorbance of the Standard solution

$C_s$  = concentration of USP Demeclocycline Hydrochloride RS in the Standard solution (mg/mL)

$L$  = label claim (mg/Tablet)

$D$  = dilution factor of the Sample solution

$V$  = volume of Medium, 900 mL



*P* = potency of demeclocycline in USP Demeclocycline Hydrochloride RS ( $\mu\text{g}/\text{mg}$ )

*F* = conversion factor,  $0.001 \text{ mg}/\mu\text{g}_{\text{USP40}}$

**Tolerances:** NLT 75% (*Q*) of the labeled amount of demeclocycline ( $\text{C}_{21}\text{H}_{21}\text{ClN}_2\text{O}_8$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## SPECIFIC TESTS

### Delete the following:

#### ▲ **LOSS ON DRYING (731)**

**Sample:** 100 mg of finely ground Tablet powder

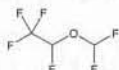
**Analysis:** Dry the *Sample* in a capillary-stoppered bottle under vacuum at  $60^\circ$  for 3 h.

**Acceptance criteria:** NMT 2% $_{\text{USP40}}$

## ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS (11)**  
USP Demeclocycline Hydrochloride RS

## Desflurane



$\text{C}_3\text{H}_2\text{F}_6\text{O}$  168.04  
Ethane, 2-(difluoromethoxy)-1,1,1,2-tetrafluoro-, ( $\pm$ );  
( $\pm$ )-2-Difluoromethyl 1,2,2,2-tetrafluoroethyl ether  
[57041-67-5].

## DEFINITION

Desflurane contains NLT 99.7% and NMT 100.0% of  $\text{C}_3\text{H}_2\text{F}_6\text{O}$ .

## IDENTIFICATION

- The IR absorption spectrum of Desflurane obtained using a gas cell exhibits maxima only at the same wavelengths as that of a similar preparation of USP Desflurane RS.

## ASSAY

### • PROCEDURE

Using the results from the *Organic Impurities* procedure, calculate the percentage of  $\text{C}_3\text{H}_2\text{F}_6\text{O}$  in the sample of Desflurane taken by subtracting the sum of all impurities found from 100.0%.

**Acceptance criteria:** 99.7%–100.0%

## IMPURITIES

### Inorganic Impurities

- **LIMIT OF NONVOLATILE RESIDUE:** Transfer 20.0 mL of Desflurane to an evaporating dish, and evaporate with a stream of nitrogen to dryness; the weight of the residue does not exceed 2.0 mg (0.01%).

### • LIMIT OF ANTIMONY

**Diluent A:** Nitric acid and water (1:1)

**Diluent B:** Nitric acid and hydrochloric acid (9:1)

**Standard solutions:** Transfer 0.1 mL (234 mg) of antimony pentachloride to a 50-mL volumetric flask, dilute with *Diluent B* to volume, and mix. This stock solution contains about 1906  $\mu\text{g}$  of antimony/mL. Dilute a portion of this solution quantitatively and stepwise with *Diluent B* to obtain *Standard solutions* containing 2.5, 5.0, and 10.0  $\mu\text{g}$  of antimony/mL.

**Sample solution:** Weigh a stoppered stock bottle containing a quantity of Desflurane at ambient temperature, and then cool it in powdered dry ice. Using a cold syringe,

transfer 5–7 mL of Desflurane from the cold bottle to a separator containing 20 mL of *Diluent A*. Allow the stock bottle containing the remaining Desflurane to come to ambient temperature, weigh it, and calculate the quantity, in g, of Desflurane taken for the test. Allow the Desflurane in the separator to evaporate, and with the aid of a few mL of *Diluent A*, transfer the acid solution to a clean, dry beaker. Add 1 mL of hydrochloric acid to the solution in the beaker, and reduce the volume to 8 mL by evaporating on a hot plate. Transfer this solution to a 10-mL volumetric flask, and dilute with *Diluent B* to volume.

## Spectrometric conditions

(See *Atomic Absorption Spectroscopy* (852).)

**Mode:** Atomic absorption spectrophotometer

**Analytical wavelength:** Antimony emission line at 217.6 nm

**Lamp:** Antimony hollow-cathode

**Flame:** Air–acetylene

**Blank:** *Diluent B*

## Analysis

**Samples:** *Standard solutions* and *Sample solution*

**Calculation:** Plot the absorbances of the *Standard solutions* versus the concentration ( $\mu\text{g}/\text{mL}$ ) of antimony, and draw the straight line best fitting the three plotted points. From the graph so obtained, determine the concentration of antimony in the *Sample solution*.

Calculate the quantity, in  $\mu\text{g}/\text{g}$ , of antimony in the portion of Desflurane taken:

$$\text{Result} = (C/W) \times V$$

*C* = concentration of antimony in the *Sample solution* ( $\mu\text{g}/\text{mL}$ )

*W* = weight of Desflurane taken to prepare the *Sample solution* (g)

*V* = volume of *Sample solution*

**Acceptance criteria:** NMT 3  $\mu\text{g}/\text{g}$

### • LIMIT OF FLUORIDE

[NOTE—Store all solutions except *Solution A* in plastic containers.]

**Solution A:** 57 mL of glacial acetic acid, 58 g of sodium chloride, and 4 g of (1,2-cyclohexylenedinitrilo)tetraacetic acid in 500 mL of water. Adjust with 5 N sodium hydroxide to a pH of  $5.25 \pm 0.25$ , and dilute with water to 1000 mL. An equivalent commercial preparation may be used.

**Standard stock solution:** 2210  $\mu\text{g}/\text{mL}$  of USP Sodium Fluoride RS in water. Each mL of this solution contains 1000  $\mu\text{g}$  of fluoride/mL.

**Standard solutions:** Dilute volumes of *Standard stock solution* with *Solution A* to obtain solutions with concentrations of 0.1, 0.3, 0.5, 1.0, 3.0, and 5.0  $\mu\text{g}$  of fluoride/mL.

**Sample solution:** Transfer 20.0 mL of Desflurane to a 60-mL separator, add 20.0 mL of water, shake for 1 min, and allow the layers to separate. Drain the lower organic layer and a small portion of the aqueous layer into a beaker, and discard. Transfer 10.0 mL of the aqueous phase remaining in the separator to a plastic cup, and add 10.0 mL of *Solution A*.

## Analysis

**Samples:** *Standard solutions* and *Sample solution*

Concomitantly measure the potentials (see *pH* (791)), in mV, of the *Samples* with a pH meter capable of a minimum reproducibility of  $\pm 0.2$  mV and equipped with a fluoride-specific ion-indicating electrode and a calomel reference electrode.

[NOTE—When taking measurements, immerse the electrodes in the solution, stir with a polytetrafluoroethylene-coated stirring bar and a magnetic stirrer having an insulated top until equilibrium is attained (about 1–2 min), and record the potential. Rinse the electrodes with *Solution A*, and dry, taking care to avoid damaging the crystal of the specific-ion electrode.]



Plot the logarithms of the fluoride concentrations ( $\mu\text{g/mL}$ ) of the *Standard solutions* versus the potential, in mV. From the measured potential of the *Sample solution* and the standard response line, determine the concentration,  $C$  ( $\mu\text{g/mL}$ ), of fluoride in the *Sample solution*. Multiply  $C$  by 0.0002 to obtain the percentage of fluoride in the portion of Desflurane taken.

**Acceptance criteria:** NMT 0.001%

### Organic Impurities

#### • PROCEDURE

**Standard stock solution:** To a suitable tared vial, fitted with a septum, add 20 mL (29.4 g) of Desflurane. Seal and re-weigh the vial to determine the weight of Desflurane added. To this vial sequentially add 20  $\mu\text{L}$  of USP Desflurane Related Compound A RS, 23  $\mu\text{L}$  of dichloromethane, 20  $\mu\text{L}$  of chloroform, 38  $\mu\text{L}$  of acetone, and 21  $\mu\text{L}$  of USP Isoflurane RS. Record the weight after the addition of each impurity and determine the total weight.

Calculate the percentage of each impurity in the *Standard stock solution*:

$$\text{Result} = W_i/W_T \times P_i$$

$W_i$  = weight of each impurity added (g)  
 $W_T$  = total weight of the *Standard stock solution* (g)  
 $P_i$  = purity of each impurity added (%)

**Standard solution:** To a suitable tared vial, fitted with a septum, add 10.2 mL (15 g) of Desflurane. Seal and re-weigh the vial to determine the weight of Desflurane added. To this vial add 250  $\mu\text{L}$  of the *Standard stock solution*, and record the weight to determine the weight of the *Standard stock solution* added and the final weight of the *Standard solution*.

Calculate the spiked concentration ( $C_s$ ) of each impurity in the *Standard solution*:

$$\text{Result} = W_i/W_T \times C_i$$

$W_i$  = weight of *Standard stock solution* added (g)  
 $W_T$  = total weight of the *Standard solution* (g)  
 $C_i$  = concentration of each impurity in the *Standard stock solution* (%)

**System suitability solution:** To a suitable vial, fitted with a septum, add 10.2 mL (15 g) of Desflurane. Seal the vial. To this vial add 100  $\mu\text{L}$  of the *Standard stock solution*.

**Sample:** Desflurane

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** GC

**Detector:** Flame ionization

**Column:** 0.32-mm  $\times$  105-m capillary column coated with 1.5- $\mu\text{m}$  film of G6

**Carrier gas:** Helium

**Autosampler/Syringe temperature:** 2°–5°

**Flow rate:** 3 mL/min

**Split flow:** 25 mL/min

**Temperature**

**Injection port:** 150°

**Detector:** 200°

**Column:** See the temperature program table below.

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
30	—	30	11
30	20	50	13

**Injection size:** 3  $\mu\text{L}$

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between isoflurane and dichloromethane, *Standard solution*

**Tailing factor:** NMT 1.5 for isoflurane, *Standard solution*

**Relative standard deviation:** NMT 5% for each impurity, *Standard solution*

**Signal-to-noise ratio:** NLT 40 for isoflurane, *System suitability solution*

#### Analysis

[NOTE—Injections of Desflurane used to prepare the *Standard solution* must be made to estimate the amount of known impurities that may be present in the solvent. The final concentration of each impurity is equal to the concentration of the impurity added plus the concentration inherent in the matrix.]

**Samples:** *Standard solution* and *Sample solution*

Calculate the final concentration of each impurity in the *Standard solution*:

$$\text{Result} = r_U/(r_S - r_U) \times C_S + C_S$$

$r_U$  = peak response of each impurity from the Desflurane used as the solvent

$r_S$  = peak response of each impurity from the *Standard solution*

$C_S$  = spiked concentration of each impurity in the *Standard solution* (%)

Calculate the percentage of each impurity observed in the *Sample solution* that is also present in the *Standard solution*:

$$\text{Result} = (r_U/r_S) \times C_F$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of each impurity from the *Standard solution*

$C_F$  = final concentration of each impurity in the *Standard solution* (%)

Calculate the percentage of all other impurities:

$$\text{Result} = (r_U/r_S) \times C_S \times 1/F$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of Isoflurane from the *Standard solution*

$C_S$  = concentration of USP Isoflurane RS in the *Standard solution* (%)

$F$  = relative response factor relative to isoflurane (see *Impurity Table 1*)

**Acceptance criteria:** See *Impurity Table 1*.

**Total impurities:** NMT 0.3%

**Impurity Table 1**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desflurane <sup>a</sup>	1.0	—	—
Dichlorofluoromethane	1.04	0.43	0.01
Trichlorofluoromethane	1.07	0.15	0.001
Desflurane related compound A <sup>b,a</sup>	1.12	—	0.10
Trichlorotrifluoroethane	1.35	1.3	0.001
Dichloromethane <sup>a</sup>	1.44	—	0.001
Isoflurane <sup>a</sup>	1.55	1.0	0.20

<sup>a</sup> These impurities are present in the *Standard solution* and are quantified by external standards.

<sup>b</sup> Bis-(1,2,2,2-tetrafluoroethyl)ether.



Impurity Table 1 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Chloroform <sup>a</sup>	1.88	—	0.006
Acetone <sup>a</sup>	2.12	—	0.010

<sup>a</sup> These impurities are present in the *Standard solution* and are quantified by external standards.

<sup>b</sup> Bis-(1,2,2,2-tetrafluoroethyl)ether.

## SPECIFIC TESTS

### • ACIDITY OR ALKALINITY

**Bromocresol purple solution:** 0.5 mg/mL of bromocresol purple. Prepared by dissolving 50 mg of bromocresol purple in 0.92 mL of 0.1 M sodium hydroxide and 20 mL of ethanol, and then diluting with water to 100 mL.

**Sample solution:** Transfer 20 mL of Desflurane to a separatory funnel, and add 20 mL of carbon dioxide-free water. Shake for 3 min, allow the layers to separate, and discard the lower organic layer. Collect the upper layer, and add 0.2 mL of *Bromocresol purple solution*.

**Acceptance criteria:** NMT 0.1 mL of 0.01 M sodium hydroxide or 0.6 mL of 0.01 M hydrochloric acid is required to change the color of the indicator.

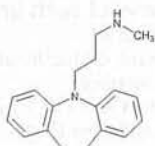
## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers. Store at controlled room temperature. Replace the cap securely after each use.

### • USP REFERENCE STANDARDS (11)

USP Desflurane RS  
USP Desflurane Related Compound A RS  
Bis-(1,2,2,2-tetrafluoroethyl)ether.  
 $C_4H_2F_8O$  218.05  
USP Isoflurane RS  
USP Sodium Fluoride RS

## Desipramine Hydrochloride



$C_{18}H_{22}N_2 \cdot HCl$  302.84  
5*H*-Dibenz[*b,f*]azepine-5-propanamine, 10,11-dihydro-*N*-methyl-, monohydrochloride;  
10,11-Dihydro-5-[3-(methylamino)propyl]-5*H*-dibenz[*b,f*]azepine monohydrochloride [58-28-6].

## DEFINITION

Desipramine Hydrochloride contains NLT 98.0% and NMT 102.0% of desipramine hydrochloride ( $C_{18}H_{22}N_2 \cdot HCl$ ).

## IDENTIFICATION

- **A. INFRARED ABSORPTION (197M)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **C. IDENTIFICATION TESTS—GENERAL, Chloride (191)**  
**Sample solution:** 50 mg/mL of Desipramine Hydrochloride in alcohol

Acceptance criteria: Meets the requirements

## ASSAY

### • PROCEDURE

**Buffer:** 3.4 g/L of sodium acetate trihydrate in water adjusted with glacial acetic acid to a pH of 5.0

**Mobile phase:** Acetonitrile, methanol, and *Buffer* (35:20:45)

**Diluent:** 0.1 M hydrochloric acid

**System suitability solution:** 0.02 mg/mL each of USP Desipramine Hydrochloride RS and USP Imipramine Hydrochloride RS in *Diluent*. Sonication may be used to promote dissolution.

**Standard solution:** 0.1 mg/mL of USP Desipramine Hydrochloride RS in *Diluent*. Sonication may be used to promote dissolution.

**Sample solution:** 0.1 mg/mL of Desipramine Hydrochloride in *Diluent*. Sonication may be used to promote dissolution.

### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 250 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L10

**Flow rate:** 1 mL/min

**Injection volume:** 25 μL

### System suitability

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for desipramine and imipramine are 1.0 and 1.1, respectively.]

### Suitability requirements

**Resolution:** NLT 1.5 between desipramine and imipramine, *System suitability solution*

**Tailing factor:** NMT 2, *Standard solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of desipramine hydrochloride ( $C_{18}H_{22}N_2 \cdot HCl$ ) in the portion of Desipramine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Desipramine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Desipramine Hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0%

## IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.1%

### Delete the following:

- **HEAVY METALS, Method II (231):** NMT 10 ppm (Official 1-Jan-2018)

### • ORGANIC IMPURITIES

**Buffer:** 5.2 g/L of dibasic potassium phosphate in water. Add 1 mL of triethylamine per L, and adjust with phosphoric acid to a pH of 6.4.

**Solution A:** Acetonitrile and methanol (55:45)

**Solution B:** *Solution A* and *Buffer* (25:75)

**Solution C:** *Solution A* and *Buffer* (62.5:37.5)

**Mobile phase:** See *Table 1*.



Table 1

Time (min)	Solution B (%)	Solution C (%)
0	85	15
35	0	100
50	0	100
50.1	85	15
60	85	15

**Standard stock solution:** 0.25 mg/mL of USP

Desipramine Hydrochloride RS prepared as follows. Transfer a suitable quantity of USP Desipramine Hydrochloride RS to an appropriate volumetric flask, and add 50% of the final flask volume of *Solution B*. Sonicate for NLT 2 min, and allow the solution to equilibrate to room temperature. Dilute with *Solution B* to volume.

**Standard solution:** 0.005 mg/mL of USP Desipramine Hydrochloride RS from *Standard stock solution* in *Solution B*

**System suitability solution:** 0.01 mg/mL each of USP Imipramine Hydrochloride RS and USP Iminodibenzyl RS in *Standard stock solution*

**Sensitivity solution:** 0.3 µg/mL of USP Desipramine Hydrochloride RS from *Standard solution* in *Solution B*. Use within 24 h.

**Sample solution:** 0.5 mg/mL of Desipramine Hydrochloride prepared as follows. Transfer a suitable quantity of Desipramine Hydrochloride to an appropriate volumetric flask, and add 50% of the final flask volume of *Solution B*. Sonicate for NLT 2 min, and allow the solution to equilibrate to room temperature. Dilute with *Solution B* to volume.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 4-µm or 5-µm packing L1

**Column temperature:** 60°

**Flow rate:** 1.4 mL/min

**Injection volume:** 40 µL

#### System suitability

**Samples:** *Standard solution*, *System suitability solution*, and *Sensitivity solution*

[NOTE—See *Table 2* for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between imipramine and iminodibenzyl, *System suitability solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Relative standard deviation:** NMT 3.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Desipramine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of desipramine from the *Standard solution*

$C_S$  = concentration of USP Desipramine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Desipramine Hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desipramine	1.0	—	—
Imipramine	1.6	1.0	0.15
Iminodibenzyl	2.1	0.55	0.1
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	1.0

#### SPECIFIC TESTS

##### • LOSS ON DRYING (731)

**Analysis:** Dry under vacuum at 105° for 2 h.

**Acceptance criteria:** NMT 0.5%

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Protect from light.

##### • USP REFERENCE STANDARDS (11)

USP Desipramine Hydrochloride RS

USP Iminodibenzyl RS

10,11-Dihydro-5H-dibenzo[b,f]azepine.

$C_{14}H_{13}N$  195.28

USP Imipramine Hydrochloride RS

## Desipramine Hydrochloride Tablets

#### DEFINITION

Desipramine Hydrochloride Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of desipramine hydrochloride ( $C_{18}H_{22}N_2 \cdot HCl$ ).

#### IDENTIFICATION

##### Delete the following:

##### ▲ A. INFRARED ABSORPTION (197M)

**Sample:** Finely powder a number of Tablets, equivalent to 350 mg of desipramine hydrochloride, and triturate the powder with 15 mL of chloroform. Pass the chloroform extract through paper into a wide-mouth test tube, and evaporate the filtrate to 3 mL. Carefully add ether until the liquid becomes turbid. Heat cautiously to produce a clear solution, then cool, and allow to stand. Collect the crystals, wash with ether, and dry under vacuum at 80° for 30 min.

**Acceptance criteria:** Meet the requirements▲USP40

##### Change to read:

- ▲A.▲USP40 The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.

##### Add the following:

- ▲ B. The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.▲USP40



## ASSAY

## Change to read:

## • PROCEDURE

**Buffer:** 3.4 g/L of sodium acetate in water. Adjust with glacial acetic acid to a pH of 5.0.

**Mobile phase:** ▲Acetonitrile, methanol,▲<sub>USP40</sub> and Buffer (30:20:50)

**Diluent:** 0.1 N hydrochloric acid

**System suitability solution:** 0.02 mg/mL each of USP Desipramine Hydrochloride RS and USP Imipramine Hydrochloride RS in *Diluent*. Sonication may be used to promote dissolution.

**Standard solution:** 0.02 mg/mL of USP Desipramine Hydrochloride RS in *Diluent*. Sonication may be used to promote dissolution.

**Sample stock solution:** Nominally 1–1.5 mg/mL of desipramine hydrochloride ▲from Tablets prepared as follows. Transfer NLT 20 Tablets▲<sub>USP40</sub> into a suitable volumetric flask. Add 50% of the final flask volume of *Diluent*. Sonicate the flask for NLT 15 min. Shake the flask for NLT 15 min. Dilute with *Diluent* to volume.

**Sample solution:** Nominally 0.02 mg/mL of desipramine hydrochloride prepared as follows. Transfer a suitable volume of *Sample stock solution* to an appropriate volumetric flask. Add 50% of the final flask volume of *Diluent*. Shake the flask for NLT 5 min and dilute with *Diluent* to volume. Pass through a suitable filter and discard the first 5 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 250 nm. ▲For *Identification B*, use a diode array detector in the range of 200–400 nm.▲<sub>USP40</sub>

**Column:** 4.6-mm × 25-cm; 5-μm packing L10

**Flow rate:** 1.5–2.0 mL/min

**Injection volume:** 25 μL

**Run time:** NLT 1.2 times the retention time of the imipramine peak

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for desipramine and imipramine are 1.0 and 1.1, respectively.]

**Suitability requirements**

**Resolution:** NLT 1.5 between desipramine and imipramine, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 1.0%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desipramine hydrochloride (C<sub>18</sub>H<sub>22</sub>N<sub>2</sub> · HCl) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

- $r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*  
 $C_S$  = concentration of USP Desipramine Hydrochloride RS in the *Standard solution* (mg/mL)  
 $C_U$  = nominal concentration of desipramine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–105.0%

## PERFORMANCE TESTS

## • DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid; 900 mL

**Apparatus 2:** 50 rpm

**Time:** 60 min

**Standard solution:** USP Desipramine Hydrochloride RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter. If necessary, dilute with *Medium* to a concentration that is similar to that of the *Standard solution*.

**Instrumental conditions**

**Mode:** UV

**Analytical wavelength:** Maximum absorbance at 251 nm

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desipramine hydrochloride (C<sub>18</sub>H<sub>22</sub>N<sub>2</sub> · HCl) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times D \times V \times (1/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Desipramine Hydrochloride RS in the *Standard solution* (mg/mL)

$D$  = dilution factor for the *Sample solution*, if needed

$V$  = volume of *Medium*, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 75% (Q) of the labeled amount of desipramine hydrochloride (C<sub>18</sub>H<sub>22</sub>N<sub>2</sub> · HCl) is dissolved.

## • UNIFORMITY OF DOSAGE UNITS (905): Meet the requirements

## IMPURITIES

## • ORGANIC IMPURITIES

**Buffer:** 5.2 g/L of dibasic potassium phosphate in water. To each L of solution, add 1 mL of triethylamine and adjust with phosphoric acid to a pH of 6.4.

**Solution A:** ▲Acetonitrile and methanol,▲<sub>USP40</sub> (55:45)

**Solution B:** *Solution A* and *Buffer* (25:75)

**Solution C:** *Solution A* and *Buffer* (62.5: 37.5)

**Mobile phase:** See *Table 1*.

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	85	15
35	0	100
50	0	100
50.1	85	15
60	85	15

**Standard stock solution:** 0.25 mg/mL of USP

Desipramine Hydrochloride RS prepared as follows. Transfer a suitable quantity of USP Desipramine Hydrochloride RS to an appropriate volumetric flask. Add 50% of the final flask volume of *Solution B*. Sonicate for NLT 2 min. Allow the solution to equilibrate to room temperature. Dilute with *Solution B* to volume.

**Standard solution:** 0.005 mg/mL of USP Desipramine Hydrochloride RS from *Standard stock solution* in *Solution B*

**System suitability solution:** 0.01 mg/mL each of USP Imipramine Hydrochloride RS and USP Iminodibenzyl RS in *Standard stock solution*

**Sensitivity solution:** 0.3 μg/mL of USP Desipramine Hydrochloride RS from *Standard solution* in *Solution B*. Use within 24 h.



**Sample solution:** Nominally 0.5 mg/mL of desipramine hydrochloride from Tablets prepared as follows. Finely powder NLT 20 Tablets. Transfer a suitable portion of this powder, equivalent to 50 mg of desipramine hydrochloride, to a 100-mL volumetric flask with the aid of *Solution B*. Add *Solution B* to about 50% of the flask volume, and sonicate the flask with occasional shaking for NLT 10 min. Allow the solution to equilibrate to room temperature. Dilute with *Solution B* to volume. Pass through a suitable filter, and discard NLT the first 2 mL of filtrate.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; 4-μm or 5-μm packing L1

**Column temperature:** 60°

**Flow rate:** 1.4 mL/min

**Injection volume:** 40 μL

#### System suitability

**Samples:** *Standard solution*, *System suitability solution*, and *Sensitivity solution*

[NOTE—See *Table 2* for relative retention times.]

#### Suitability requirements

**Resolution:** NLT 2.0 between imipramine and iminodibenzyl, *System suitability solution*

**Relative standard deviation:** NMT 3.0%, *Standard solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each degradation product in the portion of Tablets taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of each degradation product from the *Sample solution*

$r_s$  = peak response of desipramine from the *Standard solution*

$C_s$  = concentration of USP Desipramine Hydrochloride RS in the *Standard solution* (mg/mL)

$C_u$  = nominal concentration of desipramine hydrochloride in the *Sample solution* (mg/mL)

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*. Disregard peaks less than 0.05%.

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desipramine	1.0	—	—
Imipramine	1.6	1.0	0.2
Iminodibenzyl	2.1	0.55	0.5
Any unspecified degradation product	—	1.0	0.2
Total degradation products	—	—	2.0

#### ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS (11)**

USP Desipramine Hydrochloride RS

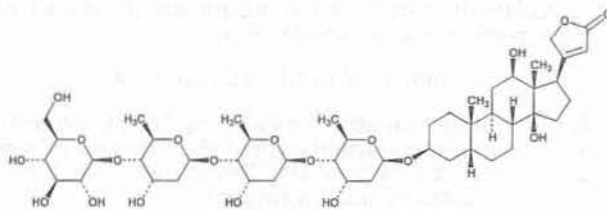
USP Iminodibenzyl RS

10,11-Dihydro-5H-dibenzo[*b,f*]azepine.

C<sub>14</sub>H<sub>13</sub>N 195.28

#### USP Imipramine Hydrochloride RS

### Deslanoside



C<sub>47</sub>H<sub>74</sub>O<sub>19</sub> 943.08

Card-20(22)-enolide, 3-[(O-β-D-glucopyranosyl-(1→4)-O-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-O-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl)oxy]-12,14-dihydroxy-, (3β,5β,12β)-; Deacetyl lanatoside C [17598-65-1].

#### DEFINITION

Deslanoside contains NLT 95.0% and NMT 103.0% of deslanoside (C<sub>47</sub>H<sub>74</sub>O<sub>19</sub>), calculated on the dried basis.

[**CAUTION**—Handle Deslanoside with exceptional care, because it is highly potent.]

#### IDENTIFICATION

##### • A. THIN-LAYER CHROMATOGRAPHY

**Standard solution:** 4 mg/mL of USP Deslanoside RS in methanol

**Sample solution:** 4 mg/mL in methanol

##### Chromatographic system

**Adsorbent:** 0.25-mm layer of chromatographic silica gel

**Application volume:** 5 μL

**Developing solvent system:** Methylene chloride, methanol, and water (130:36:3)

**Spray reagent:** Dilute perchloric acid (1 in 20)

##### Analysis

**Samples:** *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the solvent has moved three-fourths of the length of the plate. Remove the plate, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent* and heating at about 100° for 3 min. Cool, and examine under UV light.

**Acceptance criteria:** The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

#### ASSAY

##### • PROCEDURE

**Standard solution:** 0.2 mg/mL of USP Deslanoside RS in alcohol

**Sample solution:** 0.2 mg/mL of Deslanoside in alcohol

##### Instrumental conditions

**Mode:** UV-Vis

**Analytical wavelength:** 590 nm ( $\lambda_{\text{max}}$ )

**Cell:** 1 cm

**Blank:** Alcohol

##### Analysis

**Samples:** *Standard solution*, *Sample solution*, and *Blank*  
Transfer 3.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to separate 25-mL conical flasks. Evaporate each with gentle warming and with the aid of a current of air just to dryness, and cool in a vacuum desiccator for 30 min. Add 15.0 mL of acid ferric chloride TS to each flask, and mix by swirling. Allow the mixtures to stand protected from light,



swirling them frequently, at a temperature not exceeding 30°, for 15 min. Pass each solution through separate fine glass wool filters. Concomitantly determine the absorbances of the solutions, using the *Blank* to set the instrument. Repeat the measurements at 2-min intervals until maximum absorbance readings have been obtained.

Calculate the percentage of deslanoside ( $C_{47}H_{74}O_{19}$ ) in the portion of Deslanoside taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- $A_U$  = maximum absorbance of the *Sample solution*  
 $A_S$  = maximum absorbance of the *Standard solution*  
 $C_S$  = concentration of USP Deslanoside RS in the *Standard solution* (mg/mL)  
 $C_U$  = concentration of Deslanoside in the *Sample solution* (mg/mL)

Acceptance criteria: 95.0%–103.0% on the dried basis

#### IMPURITIES

- **RESIDUE ON IGNITION** (281): NMT 0.2%

#### SPECIFIC TESTS

- **OPTICAL ROTATION, Specific Rotation** (781S)  
*Sample solution*: 20 mg/mL in anhydrous pyridine  
 Acceptance criteria: +7.0° to +8.5°
- **LOSS ON DRYING** (731)  
*Sample*: 0.5 g  
*Analysis*: Dry the *Sample* under vacuum at 100° to constant weight.  
 Acceptance criteria: NMT 5.0%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in tight, light-resistant containers. Store at controlled room temperature.
- **USP REFERENCE STANDARDS** (11)  
 USP Deslanoside RS

## Deslanoside Injection

#### DEFINITION

Deslanoside Injection is a sterile solution of Deslanoside in a suitable solvent. It contains NLT 90.0% and NMT 110.0% of the labeled amount of deslanoside ( $C_{47}H_{74}O_{19}$ ). It may contain Glycerin.

#### IDENTIFICATION

##### • A. THIN-LAYER CHROMATOGRAPHY

*Standard solution*: 4 mg/mL of USP Deslanoside RS in methanol

*Sample solution*: Transfer a volume of Injection, nominally equivalent to 2 mg of deslanoside, to a small separator, and extract with 25 mL of a mixture of chloroform and alcohol (7:3). Transfer the extract to a 10-mL conical flask, evaporate on a steam bath to dryness, and dissolve the residue in 500 µL of methanol.

##### Chromatographic system

*Adsorbent*: 0.25-mm layer of chromatographic silica gel

*Application volume*: 5 µL

*Developing solvent system*: Methylene chloride, methanol, and water (130:36:3)

*Spray reagent*: Dilute perchloric acid (1 in 20)

##### Analysis

*Samples*: *Standard solution* and *Sample solution*

Allow the spots to dry, and develop the chromatogram in the *Developing solvent system* until the sol-

vent has moved three-fourths of the length of the plate. Remove the plate, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with *Spray reagent* and heating at about 100° for 3 min. Cool, and examine under UV light.

**Acceptance criteria**: The  $R_f$  value of the principal spot of the *Sample solution* corresponds to that of the *Standard solution*.

#### ASSAY

##### • PROCEDURE

*Standard solution*: 0.2 mg/mL of USP Deslanoside RS in alcohol

*Sample solution*: Transfer a volume of Injection, nominally equivalent to 600 µg of deslanoside, to a separator, and add 50 mL of water and 1 mL of 2 N sulfuric acid. Extract four 30-mL portions of a mixture of chloroform and *n*-propyl alcohol (5:1), washing each portion in a second separator containing 5 mL of water and filtering through cotton that has previously been moistened with chloroform. Combine the extracts, and evaporate on a steam bath, with the aid of a current of air, just to dryness. Transfer the residue, with the aid of a small volume of the chloroform-*n*-propyl alcohol mixture (5:1), to a 25-mL conical flask.

##### Instrumental conditions

*Mode*: UV-Vis

*Analytical wavelength*: 590 nm ( $\lambda$  max)

*Cell*: 1 cm

*Blank*: Alcohol

##### Analysis

*Samples*: *Standard solution*, *Sample solution*, and *Blank*  
 Transfer 3.0 mL each of the *Standard solution*, *Sample solution*, and *Blank* to separate 25-mL conical flasks. Evaporate each with gentle warming and with the aid of a current of air just to dryness, and cool in a vacuum desiccator for 30 min. Add 15.0 mL of acid ferric chloride TS to each flask, and mix by swirling. Allow the mixtures to stand protected from light, swirling them frequently, at a temperature not exceeding 30°, for 15 min. Pass each solution through separate fine glass wool filters. Concomitantly determine the absorbances of the solutions, using the *Blank* to set the instrument. Repeat the measurements at 2-min intervals until maximum absorbance readings have been obtained.

Calculate the percentage of deslanoside ( $C_{47}H_{74}O_{19}$ ) in the portion of Injection taken:

$$\text{Result} = (A_U/A_S) \times (C_S/C_U) \times 100$$

- $A_U$  = maximum absorbance of the *Sample solution*  
 $A_S$  = maximum absorbance of the *Standard solution*  
 $C_S$  = concentration of USP Deslanoside RS in the *Standard solution* (µg/mL)  
 $C_U$  = nominal concentration of deslanoside in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

#### SPECIFIC TESTS

- **PH** (791): 5.5–7.0
- **OTHER REQUIREMENTS**: It meets the requirements in *Injections and Implanted Drug Products* (1).

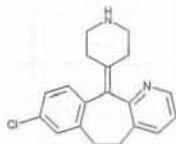
#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Preserve in single-dose containers, preferably of Type I glass.



- **USP REFERENCE STANDARDS (11)**  
USP Desloratadine RS

## Desloratadine



$C_{19}H_{19}ClN_2$  310.82  
Benzo[5,6]cyclohepta[1,2-*b*]pyridine, 8-chloro-6,11-dihydro-11-(4-piperidinylidene)-, 5*H*-;  
8-Chloro-6,11-dihydro-11-(piperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine [100643-71-8].

### DEFINITION

Desloratadine contains NLT 98.0% and NMT 102.0% of desloratadine ( $C_{19}H_{19}ClN_2$ ), calculated on the anhydrous and solvent-free basis.

### IDENTIFICATION

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** Dissolve 0.865 g of sodium dodecyl sulfate in water, add 0.5 mL of trifluoroacetic acid, and dilute with water to 1 L.

**Mobile phase:** Acetonitrile and *Buffer* (43:57)

**System suitability solution:** 0.08 mg/mL of USP Desloratadine RS and 0.2 µg/mL of USP Desloratadine Related Compound B RS in *Mobile phase*

**Standard solution:** 0.08 mg/mL of USP Desloratadine RS in *Mobile phase*

**Sample solution:** 0.08 mg/mL of Desloratadine in *Mobile phase*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 4-µm packing L1

**Column temperature** 35°

**Flow rate:** 1 mL/min

**Injection volume:** 100 µL

**Run time:** NLT 2 times the retention time of desloratadine

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between desloratadine related compound B and desloratadine, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*  
Calculate the percentage of desloratadine ( $C_{19}H_{19}ClN_2$ ) in the portion of Desloratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*  
 $r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Desloratadine in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the anhydrous and solvent-free basis

### IMPURITIES

- **RESIDUE ON IGNITION (281):** NMT 0.2%

#### • ORGANIC IMPURITIES, PROCEDURE 1

Use *Organic Impurities, Procedure 1*, when the impurity profile includes desloratadine related compound B or fluorodesloratadine.

**Buffer, Mobile phase, System suitability solution, Sample solution, and Chromatographic system:** Proceed as directed in the *Assay*.

**Standard solution:** 0.08 µg/mL of USP Desloratadine RS in *Mobile phase*

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between desloratadine related compound B and desloratadine, *System suitability solution*

**Tailing factor:** NMT 1.5, *Standard solution*

**Relative standard deviation:** NMT 10.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Desloratadine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

$r_U$  = peak response of each impurity from the *Sample solution*

$r_S$  = peak response of desloratadine from the *Standard solution*

$C_S$  = concentration of USP Desloratadine RS in the *Standard solution* (µg/mL)

$C_U$  = concentration of Desloratadine in the *Sample solution* (µg/mL)

$F$  = relative response factor (see *Table 1*)

**Acceptance criteria:** See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Fluorodesloratadine <sup>a</sup>	0.8	0.6	0.2
Desloratadine related compound B	0.9	0.6	0.3
Desloratadine	1.0	—	—
Any other individual unspecified impurity	—	1.00	0.10
Total impurities	—	—	0.4

<sup>a</sup> 8-Chloro-11-fluoro-11-(piperidin-4-yl)-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine.

#### • ORGANIC IMPURITIES, PROCEDURE 2

Use *Organic Impurities, Procedure 2*, when the impurity profile includes dechloro desloratadine, desloratadine related compound A, or dehydrodesloratadine.

**Buffer:** 1.36 g/L of monobasic potassium phosphate in water. Add 10 mL of triethylamine per L of the solution, and adjust with dilute phosphoric acid (1 in 10) to a pH of 2.0.

**Solution A:** Acetonitrile, methanol, and *Buffer* (10:10:80)

**Solution B:** Acetonitrile, tetrahydrofuran, and *Buffer* (70:5:30)



Mobile phase: See Table 2.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
15	90	10
20	70	30
25	60	40
30	50	50
38	50	50
40	100	0
45	100	0

**System suitability solution:** 0.15 µg/mL of USP Desloratadine Related Compound A RS and 100 µg/mL of USP Desloratadine RS in *Solution A*

**Standard solution:** 0.5 µg/mL of USP Desloratadine RS in *Solution A*

**Sample solution:** 500 µg/mL of Desloratadine in *Solution A*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Flow rate:** 1 mL/min

**Injection volume:** 60 µL

#### System suitability

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 3.0 between desloratadine and desloratadine related compound A, *System suitability solution*

**Tailing factor:** NMT 3.0 for desloratadine, *System suitability solution*

**Relative standard deviation:** NMT 5.0%, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Desloratadine taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times (1/F) \times 100$$

$r_u$  = peak response of each impurity from the *Sample solution*

$r_s$  = peak response of desloratadine from the *Standard solution*

$C_s$  = concentration of USP Desloratadine RS in the *Standard solution* (µg/mL)

$C_u$  = concentration of Desloratadine in the *Sample solution* (µg/mL)

$F$  = relative response factor (see Table 3)

**Acceptance criteria:** See Table 3.

Table 3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Dechloro desloratadine <sup>a</sup>	0.38	0.90	0.15
Desloratadine	1.0	—	—

<sup>a</sup> 6,11-Dihydro-11-(piperidin-4-ylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.

<sup>b</sup> 8-Chloro-11-(piperidin-4-ylidene)benzo[5,6]cyclohepta[1,2-b]pyridine.

<sup>c</sup> 8-Chloro-6,11-dihydro-11-(1-ethoxycarbonylpiperidin-4-ylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.

Table 3 (Continued)

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Desloratadine related compound A	1.30	0.86	0.15
Dehydro desloratadine <sup>b</sup>	1.59	1.00	0.15
Loratadine <sup>c</sup>	2.25	0.79	0.20
Any other individual unspecified impurity	—	1.00	0.10
Total impurities	—	—	0.40

<sup>a</sup> 6,11-Dihydro-11-(piperidin-4-ylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.

<sup>b</sup> 8-Chloro-11-(piperidin-4-ylidene)benzo[5,6]cyclohepta[1,2-b]pyridine.

<sup>c</sup> 8-Chloro-6,11-dihydro-11-(1-ethoxycarbonylpiperidin-4-ylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.

#### SPECIFIC TESTS

- **WATER DETERMINATION, Method 1c (921):** NMT 0.5%

#### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** If a test for *Organic Impurities* other than *Procedure 1* is used, then the labeling states with which *Organic Impurities* test the article complies.
- **USP REFERENCE STANDARDS (11)**
  - USP Desloratadine RS
  - USP Desloratadine Related Compound A RS
  - 8-Bromo-6,11-dihydro-11-(piperidin-4-ylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.
  - C<sub>19</sub>H<sub>19</sub>BrN<sub>2</sub> 355.27
  - USP Desloratadine Related Compound B RS
  - 8-Chloro-11-(1,2,3,6-tetrahydropyridin-4-yl)-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.
  - C<sub>19</sub>H<sub>19</sub>ClN<sub>2</sub> 310.82

## Desloratadine Tablets

#### DEFINITION

Desloratadine Tablets contain NLT 93.0% and NMT 105.0% of the labeled amount of desloratadine (C<sub>19</sub>H<sub>19</sub>ClN<sub>2</sub>).

#### IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** The UV absorption spectra of the desloratadine peak of the *Sample solution* exhibit maxima and minima at the same wavelengths as those of the corresponding peak of the *Standard solution*, as obtained in the *Assay*.

#### ASSAY

##### PROCEDURE

Use amber, low-actinic glassware.

**Buffer:** Dissolve 4.35 g of dibasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 3.0.

**Mobile phase:** Methanol and *Buffer* (20:80)

**Diluent:** Methanol and water (90:10)

**Standard solution:** 0.02 mg/mL of USP Desloratadine RS in *Diluent*

**Sample stock solution:** Nominally 0.2 mg/mL of desloratadine, prepared as follows. Transfer NLT 20 Tablets into a suitable volumetric flask, add water to fill 10% of the flask volume, and allow the Tablets to disperse. Add methanol, about 50% of the flask volume, and stir for NLT 60 min. Allow the solution to cool to room temperature and dilute with methanol to volume.



Centrifuge a portion of this solution and use the supernatant.

**Sample solution:** Nominally 0.02 mg/mL of desloratadine in *Diluent*, from *Sample stock solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

#### Detectors

Assay: UV 241 nm

Identification B: Diode array; UV 230–400 nm

Column: 4.6-mm × 15-cm; 5-μm packing L10

Column temperature: 30°

Flow rate: 1 mL/min

Injection volume: 20 μL

Run time: NLT 4.2 times the retention time of desloratadine

#### System suitability

**Sample:** *Standard solution*

#### Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desloratadine (C<sub>19</sub>H<sub>19</sub>ClN<sub>2</sub>) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of desloratadine in the *Sample solution* (mg/mL)

Acceptance criteria: 93.0%–105.0%

#### PERFORMANCE TESTS

##### • DISSOLUTION (711)

**Medium:** 0.1 N hydrochloric acid; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 45 min

**Standard solution:** 0.01 mg/mL of USP Desloratadine RS in *Medium*

**Sample solution:** Pass a portion of the solution under test through a suitable filter of 0.45-μm pore size. Discard the first 5 mL of the filtrate.

#### Instrumental conditions

Mode: UV

Analytical wavelength: 282 nm

Cell: 1.0 cm

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desloratadine (C<sub>19</sub>H<sub>19</sub>ClN<sub>2</sub>) dissolved:

$$\text{Result} = (A_U/A_S) \times C_S \times V \times (1/L) \times 100$$

$A_U$  = absorbance of the *Sample solution*

$A_S$  = absorbance of the *Standard solution*

$C_S$  = concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)

$V$  = volume of *Medium*, 500 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of desloratadine (C<sub>19</sub>H<sub>19</sub>ClN<sub>2</sub>) is dissolved.

##### • UNIFORMITY OF DOSAGE UNITS (905):

Meet the requirements

#### IMPURITIES

##### • ORGANIC IMPURITIES

**Solution A:** Dissolve 4.35 g of dibasic potassium phosphate in 1 L of water. Adjust with phosphoric acid to a pH of 3.2.

**Solution B:** Acetonitrile

**Solution C:** Methanol

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	70	15	15
12	70	15	15
30	40	30	30
45	40	30	30
47	70	15	15
55	70	15	15

**Diluent:** Methanol and water (90:10)

**Standard solution:** 0.002 mg/mL each of USP

Desloratadine RS and USP Desloratadine Related Compound F RS in *Diluent*

**Sensitivity solution:** 0.1 μg/mL of USP Desloratadine RS in *Diluent*

**Sample solution:** Nominally 0.2 mg/mL of desloratadine, prepared as follows. Transfer NLT 20 Tablets into a suitable volumetric flask, add 10% of the flask volume of water, and allow the Tablets to disperse. Add methanol, about 50% of the flask volume, and stir for at least 60 min. Allow the solution to cool to room temperature, and dilute with methanol to volume. Centrifuge a portion of this solution and use the supernatant.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 241 nm

Column: 4.6-mm × 15-cm; 3-μm packing L7

Column temperature: 35°

Flow rate: 1 mL/min

Injection volume: 20 μL

#### System suitability

**Samples:** *Standard solution* and *Sensitivity solution*

#### Suitability requirements

**Column efficiency:** NLT 1500 theoretical plates for desloratadine, *Standard solution*

**Relative standard deviation:** NMT 5.0% for desloratadine, *Standard solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

#### Analysis

**Samples:** *Standard solution*, *Sensitivity solution*, and *Sample solution*

Calculate the percentage of desloratadine related compound F in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of desloratadine related compound F from the *Sample solution*

$r_S$  = peak response of desloratadine related compound F from the *Standard solution*

$C_S$  = concentration of USP Desloratadine Related Compound F RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of desloratadine in the *Sample solution* (mg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of each unspecified impurity from the *Sample solution*

$r_S$  = peak response of desloratadine from the *Standard solution*

$C_S$  = concentration of USP Desloratadine RS in the *Standard solution* (mg/mL)



$C_U$  = nominal concentration of desloratadine in the Sample solution (mg/mL)

Acceptance criteria: See Table 2. Disregard peaks less than 0.05%.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Dechloro desloratadine <sup>a</sup>	0.37	— <sup>b</sup>
Desloratadine	1.00	—
Dehydro desloratadine <sup>c</sup>	1.4	— <sup>b</sup>
Desloratadine related compound F	1.8	0.30
Loratadine <sup>d</sup>	2.7	— <sup>b</sup>
Any unspecified degradation product	—	0.2
Total impurities	—	0.50

<sup>a</sup> 6,11-Dihydro-11-(piperidin-4-ylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.

<sup>b</sup> Process impurity controlled in the drug substance monograph. Provided for information only; the content is not calculated and not reported.

<sup>c</sup> 8-Chloro-11-(piperidin-4-ylidene)benzo[5,6]cyclohepta[1,2-b]pyridine.

<sup>d</sup> 8-Chloro-6,11-dihydro-11-(1-ethoxycarbonylpiperidin-4-ylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine (loratadine).

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers and store at controlled room temperature.
- **USP REFERENCE STANDARDS (11)**  
 USP Desloratadine RS  
 USP Desloratadine Related Compound F RS  
 8-Chloro-6,11-dihydro-11-(N-formyl-4-piperidinylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.  
 $C_{20}H_{19}ClN_2O$  338.83

## Desloratadine Orally Disintegrating Tablets

### DEFINITION

Desloratadine Orally Disintegrating Tablets contain NLT 95.0% and NMT 105.0% of the labeled amount of desloratadine ( $C_{19}H_{19}ClN_2$ ).

### IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION (197U)**  
 Standard solution and Sample solution: Proceed as directed in the Assay.  
 Instrumental conditions  
 Mode: UV  
 Wavelength range: 230–330 nm  
 [NOTE—Alternatively, a diode array detector may be used in the Assay to obtain the spectra.]  
 Acceptance criteria: The UV spectrum of the Sample solution corresponds to that of the Standard solution.
- **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

### ASSAY

#### PROCEDURE

Buffer: 6.8 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0.  
 Mobile phase: Acetonitrile, methanol, and Buffer (28:7:65)  
 Diluent: Methanol and 0.1 N hydrochloric acid (40:60)  
 Standard solution: 0.05 mg/mL of USP Desloratadine RS in Diluent. Sonication may be used to aid dissolution.  
 Sample stock solution: Nominally 0.25 mg/mL of desloratadine, prepared as follows. Transfer 10 Tablets to a suitable volumetric flask, add water to 15% of the

flask volume, and shake until the Tablets disintegrate completely. Add 75% of the flask volume of Diluent and sonicate for 30 min with intermittent shaking, and dilute with Diluent to volume. Centrifuge a portion of this solution. Use the supernatant.

Sample solution: Nominally 0.05 mg/mL of desloratadine from the Sample stock solution in Diluent; centrifuge

### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 258 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 20 μL

### System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of desloratadine ( $C_{19}H_{19}ClN_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution

$C_S$  = concentration of USP Desloratadine RS in the Standard solution (mg/mL)

$C_U$  = nominal concentration of desloratadine in the Sample solution (mg/mL)

Acceptance criteria: 95.0%–105.0%

### PERFORMANCE TESTS

- **DISINTEGRATION (701):** NMT 30 s

- **DISSOLUTION (711)**

Medium: 0.1 N hydrochloric acid (degassed); 900 mL

Apparatus 2: 50 rpm

Time: 10 min

Buffer: 6.8 g/L of monobasic potassium phosphate

Solution A: Acetonitrile and methanol (80:20)

Mobile phase: Solution A and Buffer (40:60)

Standard stock solution: 0.28 mg/mL of USP

Desloratadine RS in methanol. Sonication may be used to aid dissolution.

Standard solution: (L/900) mg/mL of USP

Desloratadine RS from the Standard stock solution in Medium, where L is the label claim (mg/Tablet)

Sample solution: Pass a portion of the solution under test through a suitable filter.

### Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 258 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1 mL/min

Injection volume: 40 μL

### System suitability

Sample: Standard solution

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

### Analysis

Samples: Standard solution and Sample solution

Calculate the percentage of the labeled amount of desloratadine ( $C_{19}H_{19}ClN_2$ ) dissolved:

$$\text{Result} = (r_U/r_S) \times C_S \times V \times (1/L) \times 100$$

$r_U$  = peak response from the Sample solution

$r_S$  = peak response from the Standard solution



$C_S$  = concentration of USP Desloratadine RS in the Standard solution (mg/mL)

$V$  = volume of Medium, 900 mL

$L$  = label claim (mg/Tablet)

**Tolerances:** NLT 80% (Q) of the labeled amount of desloratadine ( $C_{19}H_{19}ClN_2$ ) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements

## IMPURITIES

### • ORGANIC IMPURITIES

**Buffer:** Add 10 mL/L of triethylamine to a 1.36 g/L solution of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 2.5.

**Solution A:** Methanol, acetonitrile, and Buffer (15:5:80)

**Solution B:** Acetonitrile, tetrahydrofuran, and Buffer (70:5:30)

**Solution C:** Dilute 8.5 mL of hydrochloric acid with methanol to 1 L.

**Mobile phase:** See Table 1.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
10	100	0
40	50	50
50	50	50
52	100	0
65	100	0

**Diluent:** Solution C and Buffer (30:70)

**System suitability stock solution:** 0.05 mg/mL each of USP Desloratadine Related Compound A RS and USP Desloratadine Related Compound F RS in methanol

**System suitability solution:** 0.5 mg/mL of USP Desloratadine RS, 1.0 µg/mL each of USP Desloratadine Related Compound A RS and USP Desloratadine Related Compound F RS, prepared as follows. Transfer 50 mg of USP Desloratadine RS into a 100-mL volumetric flask, add 70 mL of Diluent, and sonicate to dissolve. Add 2 mL of the System suitability stock solution and dilute with Diluent to volume.

**Standard solution:** 0.0025 mg/mL of USP Desloratadine RS and 0.001 mg/mL of USP Desloratadine Related Compound F RS in Diluent

**Sample solution:** Nominally 0.5 mg/mL of desloratadine from NLT 40 Tablets, prepared as follows. Transfer an amount of powder to a suitable volumetric flask to obtain the nominal concentration of desloratadine. Add 70% of the flask volume of Mobile phase and sonicate for 20 min with intermittent shaking. Dilute with Diluent to volume. Centrifuge a portion of the solution and use the supernatant.

### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 280 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L7

**Column temperature:** 30°

**Flow rate:** 1 mL/min

**Injection volume:** 40 µL

### System suitability

**Samples:** System suitability solution and Standard solution

[NOTE—Relative retention times are given in Table 2.]

### Suitability requirements

**Resolution:** NLT 2.0 between desloratadine and desloratadine related compound A, System suitability solution

**Tailing factor:** NMT 2.0 for desloratadine and desloratadine related compound F, Standard solution

**Relative standard deviation:** NMT 10.0%

desloratadine related compound F, Standard solution

**Signal-to-noise ratio:** NLT 10 for desloratadine peak, Standard solution

### Analysis

**Samples:** Standard solution and Sample solution

Identify the impurities using the relative retention times given in Table 2.

Calculate the percentage of desloratadine related compound F in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of desloratadine related compound F from the Sample solution

$r_S$  = peak response of desloratadine related compound F from the Standard solution

$C_S$  = concentration of USP Desloratadine Related Compound F RS in the Standard solution (µg/mL)

$C_U$  = nominal concentration of desloratadine in the Sample solution (µg/mL)

Calculate the percentage of any unspecified degradation product in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of any unspecified degradation product from the Sample solution

$r_S$  = peak response of desloratadine from the Standard solution

$C_S$  = concentration of USP Desloratadine RS in the Standard solution (µg/mL)

$C_U$  = nominal concentration of desloratadine in the Sample solution (µg/mL)

**Acceptance criteria:** See Table 2.

Table 2

Compound	Relative Retention Time	Acceptance Criteria, NMT (%)
Dechloro desloratadine <sup>a,b</sup>	0.42	—
Desloratadine	1.00	—
Desloratadine related compound A <sup>b</sup>	1.09	—
Dehydro desloratadine <sup>b,c</sup>	1.33	—
Desloratadine related compound F	1.37	0.2
Loratadine <sup>b,d</sup>	1.89	—
Any other individual unspecified degradation product	—	0.20
Total degradation products	—	0.5

<sup>a</sup> 6,11-Dihydro-11-(piperidin-4-ylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.

<sup>b</sup> This is a process impurity and is included in the table for identification only. This impurity is controlled in the drug substance. It is not to be reported for the drug product and should not be included in the total impurities.

<sup>c</sup> 8-Chloro-11-(piperidin-4-ylidene)benzo[5,6]cyclohepta[1,2-b]pyridine.

<sup>d</sup> 8-Chloro-6,11-dihydro-11-(1-ethoxycarbonylpiperidin-4-ylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.

### • USP REFERENCE STANDARDS (11)

USP Desloratadine RS

USP Desloratadine Related Compound A RS

8-Bromo-6,11-dihydro-11-(piperidin-4-ylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.

$C_{19}H_{19}BrN_2$  355.27

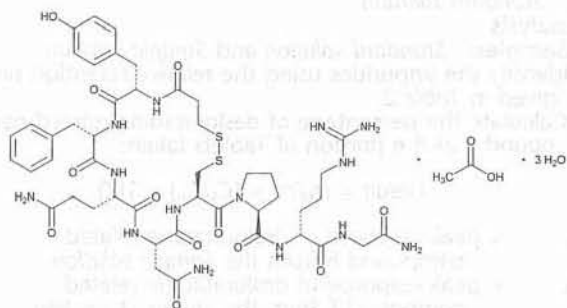
USP Desloratadine Related Compound F RS

8-Chloro-6,11-dihydro-11-(N-formyl-4-piperidinyldiene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine.

$C_{19}H_{19}ClN_2O$  338.83



## Desmopressin Acetate



$C_{48}H_{68}N_{14}O_{14}S_2 \cdot xH_2O$  (anhydrous) 1129.27  
 Anhydrous [62288-83-9].  
 Vasopressin, 1-(3-mercaptopropanoic acid)-8-D-arginine-, monoacetate (salt);  
 1-(3-Mercaptopropionic acid)-8-D-arginine-vasopressin monoacetate (salt).  
 Trihydrate 1183.31  
 [62357-86-2].

### DEFINITION

Desmopressin Acetate is a synthetic octapeptide hormone having the property of antidiuresis. It is a synthetic analog of vasopressin. It contains NLT 95.0% and NMT 105.0% of desmopressin ( $C_{46}H_{64}N_{14}O_{12}S_2$ ), calculated on the anhydrous, acetic acid-free basis.

### IDENTIFICATION

#### • A. MASS SPECTRAL ANALYSIS

**Mobile phase:** Water and methanol (1:1)

**Standard solution:** 5 µg/mL of USP Desmopressin Acetate RS in *Mobile phase*

**Sample solution:** 5 µg/mL of Desmopressin Acetate in *Mobile phase*. [NOTE—The final concentration of the *Standard solution* and the *Sample solution* can be adjusted depending on the sensitivity of the mass spectrometer used in the testing.]

#### Instrumental conditions

(See *Mass Spectrometry* (736).)

**Mode:** LC/MS spectrometer

**Interface/detection:** Infusion system connected to an electrospray interface (positive ion)

**Flow rate:** 0.7 mL/min

**Injection volume:** 10 µL/min

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Separately infuse the *Standard solution* and the *Sample solution* at about 5 µL/min into the mass spectrometer. Obtain optimized MS spectrum of the peak with mass-to-charge ratio 1069.4.

**Acceptance criteria:** MS spectra contain the major peak with mass-to-charge ratio of 1069.4.

#### • B.

**Standard solution and Sample solution:** Proceed as directed in the *Assay*.

**Identity sample solution:** 10 µg/mL of USP Desmopressin Acetate RS and 10 µg/mL of Desmopressin Acetate in *Mobile phase*

**Acceptance criteria:** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. The major peaks of the *Identity sample solution* co-elute.

### ASSAY

#### • PROCEDURE

**Buffer solution:** Dissolve 3.4 g of monobasic potassium phosphate and 2.0 g of sodium 1-heptanesulfonic acid in 1000 mL of water. Adjust with phosphoric acid or sodium hydroxide to a pH of  $4.50 \pm 0.05$ , as needed. Pass through a filter of 0.45-µm pore size.

**Mobile phase:** Acetonitrile and *Buffer solution* (22:78), and degas. Make adjustments if necessary (see *Chromatography* (621), *System Suitability*). [NOTE—The retention time of desmopressin is very sensitive to the composition of the *Mobile phase*.]

**Standard solution:** 20 µg/mL of USP Desmopressin Acetate RS in *Mobile phase*

**Sample solution:** 20 µg/mL of Desmopressin Acetate in *Mobile phase*

**System suitability solution:** Dissolve about 1 mg of oxytocin, accurately weighed, in a 50-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. Transfer 5.0 mL of the resulting solution and 5.0 mL of the *Sample solution* to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 220 nm

**Column:** 4.6-mm × 25-cm; 5-µm packing L1

**Column temperature:** 30°

**Flow rate:** 1.0 mL/min

**Injection volume:** 50 µL

#### System suitability

**Samples:** *Standard solution* and *System suitability solution*

#### Suitability requirements

**Chromatogram:** The desmopressin peak elutes before the oxytocin peak, *System suitability solution*.

**Resolution:** NLT 1.5 between desmopressin and oxytocin, *System suitability solution*

**Tailing factor:** NMT 2.0, *Standard solution*

**Relative standard deviation:** NMT 2.0% for the desmopressin peak area for replicate injections, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of desmopressin

( $C_{46}H_{64}N_{14}O_{12}S_2$ ) in the portion of Desmopressin Acetate taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Desmopressin Acetate RS (on the anhydrous, acetic acid-free basis) in the *Standard solution* (mg/mL)

$C_U$  = concentration of Desmopressin Acetate, calculated on the anhydrous, acetic acid-free basis, in the *Sample solution* (mg/mL)

**Acceptance criteria:** 95.0%–105.0% on the anhydrous, acetic acid-free basis

### IMPURITIES

#### • DESMOPRESSIN-RELATED IMPURITIES

**Mobile phase and System suitability solution:** Proceed as directed in the *Assay*.

**Standard solution:** 1 µg/mL of USP Desmopressin Acetate RS in *Mobile phase*

**Sample solution:** 200 µg/mL of Desmopressin Acetate in *Mobile phase*



**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 200 μL

**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Chromatogram:** The desmopressin peak elutes before the oxytocin peak, *System suitability solution*.**Resolution:** NLT 1.5 between desmopressin and oxytocin, *System suitability solution***Tailing factor:** NMT 2.0, *Standard solution***Relative standard deviation:** NMT 5.0% for the desmopressin peak area for replicate injections, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Record the chromatograms, and measure the response for each peak, except for the main desmopressin peak of the *Sample solution*.

Calculate the percentage of each impurity in the portion of Desmopressin Acetate taken:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

 $r_u$  = peak response of an individual impurity from the *Sample solution* $r_s$  = desmopressin peak response from the *Standard solution* $C_s$  = concentration of USP Desmopressin Acetate RS (on the anhydrous, acetic acid-free basis) in the *Standard solution* (mg/mL) $C_u$  = concentration of Desmopressin Acetate, calculated on the anhydrous, acetic acid-free basis, in the *Sample solution* (mg/mL)**Acceptance criteria**

Any individual impurity: NMT 0.5%

Total impurities: NMT 1.5%

**OTHER COMPONENTS**

- **ACETIC ACID IN PEPTIDES** (503): 3.0%–8.0%

**SPECIFIC TESTS**

- **MICROBIAL ENUMERATION TESTS** (61) and **TESTS FOR SPECIFIC MICROORGANISMS** (62): The total aerobic microbial count does not exceed 100 cfu/g.
- **WATER DETERMINATION**, *Method 1c* (921): NMT 6.0%
- **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 500 USP Endotoxin Units/mg of Desmopressin Acetate.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, preferably of Type I glass, protected from light. Store at a temperature not exceeding 25°, preferably between 2° and 8°.
- **LABELING:** Label it to state the strength, in mcg per mL, of desmopressin.
- **USP REFERENCE STANDARDS** (11)  
USP Desmopressin Acetate RS  
USP Endotoxin RS

**Desmopressin Acetate Injection****DEFINITION**

Desmopressin Acetate Injection is a sterile solution of Desmopressin Acetate in a suitable diluent. It may contain

suitable preservatives. It possesses, in each mL, an activity of NLT 90.0% and NMT 110.0% of the labeled amount of desmopressin ( $C_{46}H_{64}N_{14}O_{12}S_2$ ), calculated on the anhydrous, acetic acid-free basis.**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE****Buffer solution:** Dissolve 4.9 g of phosphoric acid, accurately weighed, in water. Dilute with water to 1000 mL, and adjust with triethylamine to a pH of 3.5.**Solution A:** Transfer 9 g of sodium chloride, accurately weighed, to a 1000-mL flask, and dissolve in and dilute with water to volume. Adjust with hydrochloric acid to a pH between 3.5 and 5.0.**Solution B:** Transfer 9 g of sodium chloride, accurately weighed, to a 1000-mL flask, dissolve in water, and add 5 g of chlorobutanol. Dilute with water to volume, and adjust with hydrochloric acid to a pH between 3.5 and 5.0.**Mobile phase:** Acetonitrile and *Buffer solution* (16.5: 83.5). Filter and degas. Make adjustments, if necessary (see *Chromatography* (621), *System Suitability*).**Sample solution:** For injections with concentrations of desmopressin between 4 μg/mL and 0.1 mg/mL, use undiluted Injection. For injections with concentrations exceeding 0.1 mg/mL and without preservatives, dilute 1000 μL of Injection, accurately measured, with 10 mL of *Solution A*. For injections with concentrations exceeding 0.1 mg/mL and containing preservatives, dilute 1000 μL of Injection, accurately measured, with 10 mL of *Solution B*.**Standard solution:** 1 mg/mL of USP Desmopressin Acetate RS in water. Dilute with *Solution A* or *Solution B*, as directed in *Sample solution*, to obtain a solution with a concentration of desmopressin equivalent to that of the *Sample solution*.**Chromatographic system**(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-μm packing L1

Flow rate: 1.5 mL/min

Injection volume: 100 μL; 50 μL for *System suitability***System suitability****Sample:** *Standard solution***Suitability requirements****Tailing factor:** NMT 1.4**Relative standard deviation:** NMT 5.0% for replicate injections**Analysis****Samples:** *Sample solution* and *Standard solution*Separately inject the *Sample solution* and the *Standard solution*, both freshly prepared, and record the chromatograms for a total time of NLT 2.5 times the retention time of the desmopressin peak.Calculate the quantity of desmopressin ( $C_{46}H_{64}N_{14}O_{12}S_2$ ), in mg, in the volume of Injection taken:

$$\text{Result} = C \times D \times (r_u/r_s)$$

 $C$  = concentration of USP Desmopressin Acetate RS in the *Standard solution* (mg/mL) $D$  = dilution factor for the *Sample solution* $r_u$  = peak response from the *Sample solution* $r_s$  = peak response from the *Standard solution***Acceptance criteria:** 90.0%–110.0% on the anhydrous, acetic acid-free basis



**SPECIFIC TESTS**

- **PH (791):** 3.5–6.0
- **BACTERIAL ENDOTOXINS TEST (85):** NMT 10 USP Endotoxin Units/μg of desmopressin
- **STERILITY TESTS (71):** It meets the requirements when tested as directed in *Test for Sterility of the Product to Be Examined, Membrane Filtration*.
- **PARTICULATE MATTER IN INJECTIONS (788):** Meets the requirements for small-volume injections
- **OTHER REQUIREMENTS:** It meets the requirements in *Injections (1)*, *Container Content*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, protected from light. Store at a temperature between 2° and 8°.
- **LABELING:** Label it to state the potency, in mg, of desmopressin.
- **USP REFERENCE STANDARDS (11)**  
USP Desmopressin Acetate RS  
USP Endotoxin RS

**Desmopressin Nasal Spray Solution****DEFINITION**

Desmopressin Nasal Spray Solution is a solution of Desmopressin Acetate in a suitable diluent. It is supplied in a form suitable for nasal administration and contains suitable preservatives. It contains NLT 90.0% and NMT 110.0% of the labeled amount of desmopressin ( $C_{46}H_{64}N_{14}O_{12}S_2$ ), calculated on the anhydrous, acetic acid-free basis.

**IDENTIFICATION**

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****• PROCEDURE**

**Buffer solution:** Dissolve 4.9 g of phosphoric acid, accurately weighed, in water. Dilute with water to 1000 mL, and adjust with triethylamine to a pH of 3.5.

**Solution A:** Transfer 9 g of sodium chloride, accurately weighed, to a 1000-mL flask. Dissolve in and dilute with water to volume. Adjust with hydrochloric acid to a pH of between 3.5 and 5.0.

**Solution B:** Transfer 9 g of sodium chloride, accurately weighed, to a 1000-mL flask, dissolve in water, and add 5 g of chlorobutanol. Dilute with water to volume, and adjust with hydrochloric acid to a pH of between 3.5 and 5.0.

**Mobile phase:** Acetonitrile and *Buffer solution* (16.5: 83.5). Filter and degas. Make adjustments, if necessary (see *Chromatography (621)*, *System Suitability*).

**Sample solution:** For nasal spray solutions with concentrations of desmopressin between 4 μg/mL and 0.1 mg/mL, use undiluted Nasal Spray Solution. For nasal spray solutions with concentrations exceeding 0.1 mg/mL and without preservatives, dilute 1000 μL of Nasal Spray Solution, accurately measured, with 10 mL of *Solution A*. For nasal spray solutions with concentrations exceeding 0.1 mg/mL and containing preservatives, dilute 1000 μL of Nasal Spray Solution, accurately measured, with 10 mL of *Solution B*.

**Standard solution:** 1 mg/mL of USP Desmopressin Acetate RS in water. Dilute with *Solution A* or *Solution B*, as directed in the *Sample solution*, to obtain a solution with a concentration of desmopressin equivalent to that of the *Sample solution*.

**Chromatographic system**

(See *Chromatography (621)*, *System Suitability*.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm × 25-cm; 5-μm packing L1

**Flow rate:** 1.5 mL/min

**Injection volume:** 100 μL; 50 μL for *System suitability*

**System suitability**

**Sample:** *Standard solution*

**Suitability requirements**

**Tailing factor:** NMT 1.4

**Relative standard deviation:** NMT 5.0% for replicate injections

**Analysis**

**Samples:** *Sample solution* and *Standard solution*

Separately inject the *Sample solution* and the *Standard solution*, both freshly prepared, and record the chromatograms for a total of NLT 2.5 times the retention time of the desmopressin peak.

Calculate the quantity of desmopressin

( $C_{46}H_{64}N_{14}O_{12}S_2$ ), in mg, in the volume of Nasal Spray Solution taken:

$$\text{Result} = C \times D \times (r_U/r_S)$$

**C** = concentration of USP Desmopressin Acetate RS in the *Standard solution* (mg/mL)

**D** = dilution factor for the *Sample solution*

**$r_U$**  = peak response from the *Sample solution*

**$r_S$**  = peak response from the *Standard solution*

**Acceptance criteria:** 90.0%–110.0% on the anhydrous, acetic acid-free basis

**SPECIFIC TESTS**

- **PH (791):** 3.5–6.0
- **MICROBIAL ENUMERATION TESTS (61) and TESTS FOR SPECIFIED MICROORGANISMS (62):** The total aerobic microbial count does not exceed 100 cfu/mL, the total combined molds and yeasts count does not exceed 10 cfu/mL, and it meets the requirements of the tests for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in containers suitable for administering the contents by spraying into the nasal cavities in a controlled, individualized dosage. Protect from light, and store at a temperature between 2° and 8°.

- **LABELING:** Label it to indicate that it is for intranasal administration only and to state the total number of discharges. Label it also to state that the dosage regulation is described in the package insert.

- **UNIFORMITY OF UNIT SPRAY WEIGHT AND TOTAL NUMBER OF DISCHARGES PER CONTAINER**

**Samples:** Three Nasal Spray Solution units

**Analysis:** Prime each spray pump as directed on the label, but NMT 5 times. Accurately weigh, by difference, 10 individual deliveries from each unit, weighing the first 3 discharges immediately after priming, 4 discharges from the middle of each unit, and 3 discharges close to the end of each unit. Continue to discharge until the unit is empty. For each unit, determine the total number of discharges, including the number of priming deliveries, and calculate the mean weight delivered per discharge.

**Acceptance criteria:** Each unit contains NLT the number of discharges stated on the label; the mean weight delivered per discharge is within 10% of the labeled weight per discharge; and NLT 9 tested discharges for each unit are between 85% and 125% of the labeled weight per discharge.



- **USP REFERENCE STANDARDS (11)**  
USP Desmopressin Acetate RS

## Desogestrel and Ethinyl Estradiol Tablets

### DEFINITION

Desogestrel and Ethinyl Estradiol Tablets contain NLT 90.0% and NMT 110.0% of the labeled amounts of desogestrel ( $C_{22}H_{30}O$ ) and ethinyl estradiol ( $C_{20}H_{24}O_2$ ).

### IDENTIFICATION

#### • A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST (201)

**Standard solution:** 0.15 mg/mL of USP Desogestrel RS and 0.03 mg/mL of USP Ethinyl Estradiol RS in ether

**Sample solution:** Transfer a number of Tablets, equivalent to 1.5 mg desogestrel and 0.3 mg ethinyl estradiol, to a suitable container, add 50 mL of water, and sonicate until the Tablets disintegrate (if necessary, remove any coating with water before sonication). Place the sample in a separatory funnel, add 25 mL of ether, and shake well to extract the actives. Using a glass pipet, transfer the ether layer to a clean beaker, and evaporate to about 10 mL.

#### Chromatographic system

(See Chromatography (621), Thin-Layer Chromatography.)

**Mode:** TLC

**Application volume:** 30  $\mu$ L

**Developing solvent system:** Chloroform and alcohol (96:4)

**Spray reagent:** Methanol and sulfuric acid (1:1)

**Analysis:** Proceed as directed in the chapter, and then air-dry. Spray the plate with the *Spray reagent*, place in an oven at 105° for about 5 min, and examine the plate.

**Acceptance criteria:** Meet the requirements

- **B.** The retention times of the major peaks of the *Sample solution* correspond to those of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### • PROCEDURE

**Buffer:** 20 mM potassium phosphate buffer, pH 6.0

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Diluent:** Acetonitrile and water (1:1)

**Standard stock solution A:** 0.3 mg/mL of USP Desogestrel RS in methanol

**Standard stock solution B:** 0.3 mg/mL of USP Ethinyl Estradiol RS in methanol

**Standard solution:** 0.6  $\mu$ g/mL of USP Desogestrel RS and 0.12  $\mu$ g/mL of USP Ethinyl Estradiol RS in *Diluent*, prepared by diluting appropriate aliquots of *Standard stock solution A* and *Standard stock solution B* with *Diluent*

**Sample solution:** Transfer 20 Tablets into a 200-mL volumetric flask. Add about 120 mL of *Diluent*, and shake for about 30 min. Dilute with *Diluent* to volume, and mix. Centrifuge a portion of the sample, and dilute with *Diluent* to obtain a solution nominally containing 0.6  $\mu$ g/mL of desogestrel.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

#### Detectors

**Desogestrel analysis:** UV 210 nm

**Ethinyl estradiol analysis:** Spectrofluorometric detector, excitation at 285 nm and emission at 310 nm

### Columns

**Guard:** 4.6-mm  $\times$  12.5-mm; packing L11

**Analytical:** 4.6-mm  $\times$  15-cm; packing L11

**Flow rate:** 2 mL/min

**Injection volume:** 200  $\mu$ L

### System suitability

**Sample:** *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol and desogestrel are about 0.2 and 1.0, respectively.]

### Suitability requirements

**Tailing factor:** NMT 2.0 for both ethinyl estradiol and desogestrel

**Relative standard deviation:** NMT 2.0%

### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desogestrel ( $C_{22}H_{30}O$ ) and ethinyl estradiol ( $C_{20}H_{24}O_2$ ) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response of the relevant analyte from the *Sample solution*

$r_S$  = peak response of the relevant analyte from the *Standard solution*

$C_S$  = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of the relevant analyte in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### PERFORMANCE TESTS

#### • DISSOLUTION (711)

##### Test 1

**Medium:** 0.05% sodium lauryl sulfate with an assay content of NLT 95%; 500 mL

**Apparatus 2:** 50 rpm

**Time:** 30 min

**Buffer:** 20 mM potassium phosphate buffer, pH 6.0

**Mobile phase:** Acetonitrile and *Buffer* (1:1)

**Standard stock solution A:** 0.005 mg/mL of USP Desogestrel RS in *Medium* prepared as follows. Dissolve a sufficient quantity of USP Desogestrel RS in methanol to obtain a solution containing 0.25 mg/mL of USP Desogestrel RS. Dilute 1.0 mL of this solution with *Medium* to 50.0 mL.

**Standard stock solution B:** 0.005 mg/mL of USP Ethinyl Estradiol RS in *Medium* prepared as follows. Dissolve a sufficient quantity of USP Ethinyl Estradiol RS in methanol to obtain a solution containing 0.25 mg/mL of USP Ethinyl Estradiol RS. Dilute 1.0 mL of this solution with *Medium* to 50.0 mL.

**Standard solution:** 0.3  $\mu$ g/mL of USP Desogestrel RS and 0.06  $\mu$ g/mL of USP Ethinyl Estradiol RS in *Medium*, from *Standard stock solution A* and *Standard stock solution B*

**Sample solution:** Sample per *Dissolution (711)*. Centrifuge a portion of the dissolution sample, and use the clear supernatant.

#### Chromatographic system

(See Chromatography (621), System Suitability.)

**Mode:** LC

#### Detectors

**Desogestrel analysis:** UV 210 nm

**Ethinyl estradiol analysis:** Spectrofluorometric detector, excitation at 285 nm and emission at 310 nm



**Columns**

Guard: 4.6-mm × 12.5-mm; packing L11

Analytical: 4.6-mm × 15-cm; packing L11

Flow rate: 2 mL/min

Injection volume: 200 µL

**System suitability**Sample: *Standard solution*

[NOTE—The relative retention times for ethinyl estradiol and for desogestrel are about 0.2 and 1.0, respectively.]

**Suitability requirements**

Relative standard deviation: NMT 3.0%

**Analysis**Samples: *Standard solution* and *Sample solution*Determine the amounts of desogestrel (C<sub>22</sub>H<sub>30</sub>O) and ethinyl estradiol (C<sub>20</sub>H<sub>24</sub>O<sub>2</sub>) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

$r_U$  = peak response of the relevant analyte from the *Sample solution*

$r_S$  = peak response of the relevant analyte from the *Standard solution*

$C_S$  = concentration of the appropriate USP Reference Standard in the *Standard solution* (mg/mL)

$L$  = label claim (mg/Tablet)

$V$  = volume of medium, 500 mL

**Tolerances:** NLT 80% (Q) of each of the labeled amounts of desogestrel (C<sub>22</sub>H<sub>30</sub>O) and ethinyl estradiol (C<sub>20</sub>H<sub>24</sub>O<sub>2</sub>) is dissolved.

**Test 2:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

**Medium:** 0.3% sodium lauryl sulfate; 500 mL

**Apparatus 2:** 100 rpm

**Time:** 30 min

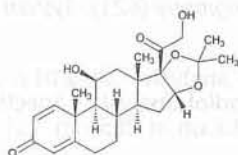
**Analysis:** Determine the amounts of desogestrel (C<sub>22</sub>H<sub>30</sub>O) and ethinyl estradiol (C<sub>20</sub>H<sub>24</sub>O<sub>2</sub>) dissolved by the chromatographic method used in *Test 1*.

**Tolerances:** NLT 80% (Q) of each of the labeled amounts of desogestrel (C<sub>22</sub>H<sub>30</sub>O) and ethinyl estradiol (C<sub>20</sub>H<sub>24</sub>O<sub>2</sub>) is dissolved.

- **UNIFORMITY OF DOSAGE UNITS (905):** Meet the requirements for *Content Uniformity* for both desogestrel and ethinyl estradiol

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP REFERENCE STANDARDS (11)**  
USP Desogestrel RS  
USP Ethinyl Estradiol RS

**Desonide**C<sub>24</sub>H<sub>32</sub>O<sub>6</sub>

416.51

Pregna-1,4-diene-3,20-dione, 11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-, (11β,16α)-;

11β,16α,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone [638-94-8].

**DEFINITION**

Desonide contains NLT 98.0% and NMT 102.0% of the labeled amount of desonide (C<sub>24</sub>H<sub>32</sub>O<sub>6</sub>), calculated on the dried basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION (197K)**
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

**ASSAY****PROCEDURE**

Use low-actinic glassware for all solutions containing desonide.

**Solution A:** 0.1% Phosphoric acid in water

**Solution B:** Acetonitrile

**Mobile phase:** See *Table 1*.

**Table 1**

Time (min)	Solution A (%)	Solution B (%)
0	70	30
4	68	32
5	68	32
9	65	35
13	30	70
16	30	70

**Diluent:** *Solution A* and acetonitrile (60:40)

**System suitability solution:** 0.7 mg/mL of USP Desonide Impurities Mixture RS in *Diluent*

**Standard solution:** 0.7 mg/mL of USP Desonide RS in *Diluent*

**Sample solution:** 0.7 mg/mL of Desonide in *Diluent*

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 15-cm; 2.6-µm packing L7

**Temperatures**

**Column:** 20° (15°–22° was shown to be acceptable)

**Autosampler:** 20°

**Flow rate:** 1 mL/min

**Injection volume:** 5 µL

**System suitability**

**Samples:** *System suitability solution* and *Standard solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between desonide glyoxal and deoxyprednisolone-16-ene; NLT 2.0 between desonide and dihydrodesonide, *System suitability solution*

**Relative standard deviation:** NMT 0.73%, *Standard solution*

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of desonide (C<sub>24</sub>H<sub>32</sub>O<sub>6</sub>) in the portion of Desonide taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

$r_U$  = peak response from the *Sample solution*

$r_S$  = peak response from the *Standard solution*

$C_S$  = concentration of USP Desonide RS in the *Standard solution* (mg/mL)

$C_U$  = concentration of Desonide in the *Sample solution* (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the dried basis

**IMPURITIES**

- **RESIDUE ON IGNITION (281):** NMT 0.10%

**ORGANIC IMPURITIES**

*Solution A*, *Solution B*, *Mobile phase*, *Diluent*, *System suitability solution*, *Standard solution*, *Sample solu-*



**tion, and Chromatographic system:** Proceed as directed in the *Assay*.

**Sensitivity solution:** 0.28 µg/mL of USP Desonide RS in Diluent from the *Standard solution*

**System suitability**

**Samples:** *System suitability solution* and *Sensitivity solution*

**Suitability requirements**

**Resolution:** NLT 2.0 between desonide glyoxal and deoxyprednisolone-16-ene; NLT 2.0 between desonide and dihydrodesonide, *System suitability solution*

**Signal-to-noise ratio:** NLT 10, *Sensitivity solution*

**Analysis**

**Sample:** *Sample solution*

Calculate the percentage of each impurity in the portion of Desonide taken:

$$\text{Result} = (r_u/r_T) \times (1/F) \times 100$$

$r_u$  = peak area of each impurity from the *Sample solution*

$r_T$  = total peak area from the *Sample solution*

$F$  = relative response factor (see *Table 2*)

**Acceptance criteria:** See *Table 2*.

[NOTE—Disregard peaks that are less than 0.04% of the desonide peak from the *Standard solution*.]

**Table 2**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
16α-Hydroxyprednisolone <sup>a</sup>	0.31	1.0	0.15
Prednisolone <sup>b</sup>	0.48	1.0	0.15
Desonide glyoxal <sup>c</sup>	0.76	1.0	0.50
Deoxyprednisolone-16-ene <sup>d</sup>	0.82	1.7	0.50
Sum of desonide glyoxal and deoxyprednisolone-16-ene	—	—	0.50
Desonide	1.00	—	—
Dihydrodesonide <sup>e</sup>	1.07	0.84	0.50
Epoxydesonide <sup>f</sup>	1.18	1.0	0.50
Sum of dihydrodesonide and epoxydesonide	—	—	0.50
Bromodesonide <sup>g</sup>	1.43	0.68	0.15
Acetylidesonide <sup>h</sup>	1.74	0.82	0.15

<sup>a</sup> 11β,16α,17,21-Tetrahydroxy-3,20-dioxopregna-1,4-diene.

<sup>b</sup> 11β,17,21-Trihydroxy-3,20-dioxopregna-1,4-diene.

<sup>c</sup> 11β-Hydroxy-16α,17-[(1-methylethylidene)bis(oxy)]-3,20-dioxopregna-1,4-dien-21-al.

<sup>d</sup> 11β,21-Dihydroxypregna-1,4,16-triene-3,20-dione.

<sup>e</sup> 11β,21-Dihydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregn-4-ene-3,20-dione.

<sup>f</sup> 9,11β-Epoxy-21-hydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione.

<sup>g</sup> 9α-Bromo-11β,21-dihydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-dione.

<sup>h</sup> 11β-Hydroxy-16α,17-[(1-methylethylidene)bis(oxy)]-3,20-dioxopregna-1,4-dien-21-yl acetate.

**Table 2 (Continued)**

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Any other impurity	—	1.0	0.10
Total impurities	—	—	1.0

<sup>a</sup> 11β,16α,17,21-Tetrahydroxy-3,20-dioxopregna-1,4-diene.

<sup>b</sup> 11β,17,21-Trihydroxy-3,20-dioxopregna-1,4-diene.

<sup>c</sup> 11β-Hydroxy-16α,17-[(1-methylethylidene)bis(oxy)]-3,20-dioxopregna-1,4-dien-21-al.

<sup>d</sup> 11β,21-Dihydroxypregna-1,4,16-triene-3,20-dione.

<sup>e</sup> 11β,21-Dihydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregn-4-ene-3,20-dione.

<sup>f</sup> 9,11β-Epoxy-21-hydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione.

<sup>g</sup> 9α-Bromo-11β,21-dihydroxy-16α,17-[(1-methylethylidene)bis(oxy)]pregna-1,4-diene-3,20-dione.

<sup>h</sup> 11β-Hydroxy-16α,17-[(1-methylethylidene)bis(oxy)]-3,20-dioxopregna-1,4-dien-21-yl acetate.

## SPECIFIC TESTS

### • LOSS ON DRYING (731)

**Sample:** 1.0 g

**Analysis:** Dry at 105° for 3 h.

**Acceptance criteria:** NMT 0.5%

### • OPTICAL ROTATION, Specific Rotation (781S)

**Sample:** 10 mg/mL in dioxane

**Acceptance criteria:** +104.0° to +110.0°, calculated on the dried basis

## ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature. Protect from light.

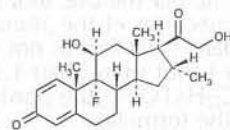
### • USP REFERENCE STANDARDS (11)

USP Desonide RS

USP Desonide Impurities Mixture RS

This is a mixture that contains desonide and may contain about 1% each of 16α-hydroxyprednisolone, prednisolone, desonide glyoxal, deoxyprednisolone-16-ene, dihydrodesonide, epoxydesonide, bromodesonide, and acetylidesonide.

## Desoximetasone



C<sub>22</sub>H<sub>29</sub>FO<sub>4</sub> 376.46

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16-methyl-, (11β,16α)-.

9-Fluoro-11β,21-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione [382-67-2].

» Desoximetasone contains not less than 97.0 percent and not more than 103.0 percent of C<sub>22</sub>H<sub>29</sub>FO<sub>4</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards (11)**—

USP Desoximetasone RS

**Identification**—

**A: Infrared Absorption (197K).**

**B:** Prepare a solution of it in a mixture of chloroform and alcohol (3:1) containing 10 mg per mL. Prepare a solution



of USP Desoximetasone RS in the same mixture, containing 10 mg per mL. Apply separately 20  $\mu$ L of each solution to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the chromatogram in a saturated chamber containing a mixture of chloroform and ethyl acetate (1:1). Allow the solvent front to move 10 cm beyond the application point. After drying, examine the plate under UV light at 254 nm. Spray the dried plate with a 1 in 5 solution of *p*-toluenesulfonic acid in alcohol. The major spot from the test solution corresponds in  $R_f$  value (about 0.25) and appearance to that obtained from the Standard solution.

**Melting range** (741): between 206° and 218°, but the range between beginning and end of melting does not exceed 4°.

**Specific rotation** (781S): between +107° and +112°.

*Test solution:* 5 mg per mL, in chloroform.

**Loss on drying** (731)—Dry it at 105° to constant weight; it loses not more than 1.0% of its weight.

**Residue on ignition** (281): not more than 0.2%.

#### Delete the following:

• **Heavy metals, Method II** (231): 0.002%. (Official 1-Jan-2018)

#### Assay—

**Mobile phase**—Prepare a suitable filtered and degassed solution of methanol, water, and glacial acetic acid (65:35:1), such that the retention time of desoximetasone is about 6 minutes.

**Standard preparation**—On the day of use, weigh accurately about 20 mg of USP Desoximetasone RS, and dissolve in methanol to obtain 50.0 mL. Dilute 10.0 mL of this solution with a mixture of methanol and acetonitrile (1:1) to 100.0 mL.

**Assay preparation**—Weigh accurately 40 mg of Desoximetasone, dissolve in 100.0 mL of methanol, and proceed as directed for *Standard preparation*, beginning with "Dilute 10.0 mL of this solution."

**Procedure**—Using an injection loop, inject 10- $\mu$ L portions of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph equipped with an UV detector capable of monitoring absorption at 254 nm. The instrument is equipped with a 4.6-mm  $\times$  15-cm stainless steel column that contains packing L7 and is operated at a flow rate of about 1 mL per minute. In a suitable chromatogram, five replicate injections of the *Standard preparation* show a relative standard deviation of not more than 2.0%, and the tailing factor is not more than 1.5. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_4$  in the portion of Desoximetasone taken by the formula:

$$1000C(H_U / H_S)$$

in which *C* is the concentration, in mg per mL, of USP Desoximetasone RS in the *Standard preparation*, and  $H_U$  and  $H_S$  are the peak heights of desoximetasone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Desoximetasone Cream

» Desoximetasone Cream is Desoximetasone in an emollient cream base. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{29}FO_4$ .

**Packaging and storage**—Preserve in collapsible tubes, at controlled room temperature.

#### USP Reference standards (11)—

USP Desoximetasone RS

**Identification**—Evaporate 25 mL of the *Assay preparation*, prepared as directed in the *Assay*, on a steam bath just to dryness, and dissolve the residue in 2 mL of acetonitrile. This is the test solution. Prepare a Standard solution of USP Desoximetasone RS in acetonitrile containing 500  $\mu$ g per mL. Using 10  $\mu$ L instead of 20  $\mu$ L of each solution, proceed as directed in *Identification test B* under *Desoximetasone*, beginning with "Apply separately 20  $\mu$ L of each." The specified result is observed.

**Minimum fill** (755): meets the requirements.

**pH** (791): between 4.0 and 8.0, in a solution prepared in the following manner. Add 15 mL of boiling water to 3.5 g of the Cream in a 50-mL centrifuge tube, cap the tube, shake vigorously until the cream is uniformly dispersed, then place the tube in a steam bath until the water and oil layers separate completely. Cool, separate the layers, and determine the pH of the aqueous phase.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, water, and glacial acetic acid (65:35:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Internal standard solution**—Prepare a solution of ethylparaben in methanol having a concentration of about 0.04 mg per mL.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Desoximetasone RS in methanol to obtain a solution having a known concentration of about 0.4 mg per mL. Pipet 5 mL of this solution into a 50-mL centrifuge tube. Add 10.0 mL of *Internal standard solution*, dilute with methanol quantitatively to 40.0 mL, and mix to obtain the *Standard preparation* having a known concentration of about 0.05 mg per mL.

**Assay preparation**—Transfer an accurately weighed amount of Cream, equivalent to about 2 mg of desoximetasone, to a 50-mL centrifuge tube, and add a few 3-mm glass beads. Add 10.0 mL of *Internal standard solution* and about 30 mL of methanol, and mix. Tightly cap the centrifuge tube, and immerse it for 10 minutes in a bath maintained at a temperature of 65°. Remove the tube from the bath, and immediately vortex at high speed for 30 seconds. Return the tube to the hot water bath for 5 minutes, remove it from the bath, and immediately vortex for 30 seconds. Repeat the procedure one more time, then cool the tube in an ice-bath held at 10° until no further flocculent precipitation occurs. Centrifuge, and use the supernatant.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative retention times are about 1 and 2 for ethylparaben and desoximetasone, respectively; the tailing factor for the analyte peak is not more than 2.0; the resolution, *R*, between the analyte and internal standard peaks is not less than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_4$  in the portion of Cream taken by the formula:

$$40C(R_U/R_S)$$

in which *C* is the concentration, in mg per mL, of USP Desoximetasone RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.



## Desoximetasone Gel

» Desoximetasone Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{29}FO_4$ .

**Packaging and storage**—Preserve in collapsible tubes, at controlled room temperature.

### USP Reference standards (11)—

USP Desoximetasone RS

**Identification**—Transfer an amount of Gel, equivalent to 100  $\mu$ g of desoximetasone, to a 15-mL centrifuge tube. Add 3 mL of acetonitrile, sonicate for approximately 1 minute, centrifuge, and transfer the clear supernatant to another 15-mL centrifuge tube. Evaporate the solution under nitrogen at a temperature between 35° and 45° to dryness. Dissolve the residue in 100  $\mu$ L of methanol, using a sonicator. Streak separately the entire test solution and 100  $\mu$ L of a Standard solution of USP Desoximetasone RS in methanol containing 1 mg per mL on a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture and an area of preadsorbent material on which specimens are applied. Allow the streaks to dry, and develop the chromatogram in a saturated chamber containing a mixture of acetone and chloroform (1:1). Allow the solvent front to move not less than 10 cm beyond the origin. After drying, examine the plate under UV light at 254 nm: the  $R_f$  value of the principal spot obtained in the chromatogram of the test solution corresponds to that of the Standard solution.

**Minimum fill** (755): meets the requirements.

**Alcohol content**—Transfer about 2.5 g of Gel, accurately weighed, to a 50-mL volumetric flask. Dissolve in methanol, dilute with methanol to volume, and mix. Determine the alcohol content of the specimen thus prepared by the *Method II—Gas-Liquid Chromatographic Method* (see *Alcohol Determination* (611)), using isopropyl alcohol as the internal standard and using methanol in place of water as the solvent: between 18.0% and 24.0% (w/w) of  $C_2H_5OH$  is found.

### Assay—

**Mobile phase**—Prepare a suitable filtered and degassed solution of methanol, water, and glacial acetic acid (65:35:1). Adjust the ratio, if necessary, so that the retention time of desoximetasone is about 8 minutes.

**Standard preparation**—Using an accurately weighed quantity of USP Desoximetasone RS, prepare a solution in methanol containing 0.5 mg per mL. Dilute an accurately measured volume of this solution with methanolic calcium chloride dihydrate solution (1.5 in 100) to obtain a *Standard preparation* having a known concentration of about 0.025 mg per mL.

**Assay preparation**—Transfer an accurately weighed quantity of Gel, equivalent to about 1.25 mg of desoximetasone, to a 50-mL volumetric flask, add approximately 40 mL of methanolic calcium chloride dihydrate solution (1.5 in 100), and sonicate to disperse the gel. Dilute with the same solution to volume, mix, and centrifuge. Use the clear supernatant.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph five replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph by means of a sampling valve, record the chromatograms, and measure the responses for the ma-

jor peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_4$  in the portion of Gel taken by the formula:

$$50C(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Desoximetasone RS in the *Standard preparation*; and  $r_U$  and  $r_S$  are the peak responses of desoximetasone obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Desoximetasone Ointment

» Desoximetasone Ointment contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of desoximetasone ( $C_{22}H_{29}FO_4$ ).

**Packaging and storage**—Preserve in collapsible tubes, at controlled room temperature.

### USP Reference standards (11)—

USP Desoximetasone RS

**Identification**—Transfer an accurately weighed quantity of Ointment, equivalent to about 5 mg of desoximetasone, to a 50-mL centrifuge tube. Add 20 mL of hexane, heat gently to 60°, and shake until the Ointment is completely dispersed. Add 8 mL of acetonitrile, insert the stopper in the tube, and shake vigorously for 5 minutes. Cool to room temperature, and centrifuge until the lower layer is clear. Transfer the lower layer to a 10-mL volumetric flask, dilute with acetonitrile to volume, and mix. Prepare a solution of USP Desoximetasone RS in acetonitrile containing 0.5 mg per mL. Separately apply 5  $\mu$ L of each solution to a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Allow the spots to dry, and develop the plate in a saturated chamber containing a mixture of ethyl acetate and chloroform (4:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate, and allow to air-dry. Examine under short-wavelength UV light. Spray the dried plate with a 1 in 5 solution of *p*-toluenesulfonic acid in alcohol. Heat the plate at 100° for 5 minutes, and examine under long-wavelength UV light: the  $R_f$  value and appearance (brownish yellow fluorescent spot) of the principal spot from the test solution, correspond to those of the principal spot from the *Standard solution*.

**Minimum fill** (755): meets the requirements.

### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol, water, and glacial acetic acid (65:35:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Desoximetasone RS in methanol to obtain a solution having a known concentration of about 0.4 mg per mL. Quantitatively dilute 1 volume of this solution with 9 volumes of a 1:1 mixture of methanol and spectrophotometric acetonitrile that is saturated with *n*-heptane, and mix.

**Assay preparation**—Transfer an accurately weighed amount of Ointment, equivalent to about 2 mg of desoximetasone, to a 50-mL centrifuge tube. Add 20 mL of *n*-heptane that has been previously saturated with spectrophotometric acetonitrile, and heat gently with occasional shaking until the Ointment is completely dispersed. Allow to cool slightly, and extract with a 10-mL portion of spectrophotometric acetonitrile. Shake vigorously, centrifuge, remove the bottom layer of acetonitrile with a syringe and



needle, and transfer to a 50-mL volumetric flask. Using the same needle and syringe, extract the desoximetasone with successive 10-mL and 8-mL portions of acetonitrile, combining all acetonitrile layers in the 50-mL flask. Dilute with methanol nearly to volume, mix, and allow the solution to reach room temperature. Dilute with methanol to volume, and mix.

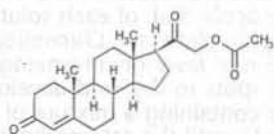
**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 15-cm column that contains packing L7. The flow rate is about 1 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the tailing factor for the analyte peak is not more than 2.0, the resolution, *R*, between the analyte and solvent peaks is not less than 5.0, and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 10 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_4$  in the portion of Ointment taken by the formula:

$$50C(r_U / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Desoximetasone RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Desoxycorticosterone Acetate



$C_{23}H_{32}O_4$  372.50  
Pregn-4-ene-3,20-dione, 21-(acetyloxy)-  
11-Deoxycorticosterone acetate [56-47-3].

» Desoxycorticosterone Acetate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{23}H_{32}O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**USP Reference standards** (11)—  
USP Desoxycorticosterone Acetate RS

### Identification—

**A:** Infrared Absorption (197K).

**B:** Ultraviolet Absorption (197U)—

*Solution:* 10 µg per mL.

*Medium:* alcohol.

Absorptivities at 240 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 155° and 161°.

**Specific rotation** (781S): between +171° and +179°.

*Test solution:* 10 mg, undried, per mL, in dioxane.

**Loss on drying** (731)—Dry it in vacuum over silica gel for 4 hours: it loses not more than 0.5% of its weight.

### Assay—

**Standard preparation**—Prepare as directed for *Standard Preparation* under *Assay for Steroids* (351), using USP Desoxycorticosterone Acetate RS.

**Assay preparation**—Accurately weigh about 100 mg of Desoxycorticosterone Acetate, dissolve in sufficient alcohol to make 200.0 mL, and mix. Pipet 5 mL of this solution into a 250-mL volumetric flask, add alcohol to volume, and mix. Pipet 20 mL of the resulting solution into a glass-stoppered, 50-mL conical flask.

**Procedure**—Proceed as directed for *Procedure* under *Assay for Steroids* (351). Calculate the quantity, in mg, of  $C_{23}H_{32}O_4$  in the Desoxycorticosterone Acetate taken by the formula:

$$10C(A_U / A_S)$$

## Desoxycorticosterone Acetate Injection

» Desoxycorticosterone Acetate Injection is a sterile solution of Desoxycorticosterone Acetate in vegetable oil. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amount of  $C_{23}H_{32}O_4$ .

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I or Type III glass, protected from light.

**USP Reference standards** (11)—

USP Desoxycorticosterone Acetate RS

USP Endotoxin RS

**Identification**—Evaporate 25 mL of the *Assay preparation* from the *Assay* on a steam bath just to dryness, and dissolve the residue in 1 mL of chloroform. Using this as the test solution, proceed as directed under *Thin-Layer Chromatographic Identification Test* (201).

**Bacterial Endotoxins Test** (85)—It contains not more than 71.4 USP Endotoxin Units per mg of desoxycorticosterone acetate.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

### Assay—

**Alcohol-isooctane and Isooctane-alcohol**—Shake equal volumes of 90 percent alcohol and isooctane in a separator for 10 to 15 minutes, and allow to separate. Withdraw the layers into separate containers, designating the lower layer as "alcohol-isooctane" and the upper layer as "isooctane-alcohol."

**Standard preparation**—Prepare as directed for *Standard Preparation* under *Assay for Steroids* (351), using USP Desoxycorticosterone Acetate RS.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 5 mg of desoxycorticosterone acetate, to a separator containing 50 mL of *Isooctane-alcohol*. Extract with six 20-mL portions of *Alcohol-isooctane*, receiving the extracts in a 250-mL volumetric flask, dilute with *Alcohol-isooctane* to volume, and mix. Pipet 10 mL of this solution into a glass-stoppered, 50-mL conical flask, evaporate on a steam bath with the aid of a gentle current of air just to dryness, and dissolve the residue in 20.0 mL of alcohol.

**Procedure**—Proceed as directed for *Procedure* under *Assay for Steroids* (351). Calculate the quantity, in mg, of  $C_{23}H_{32}O_4$  in each mL of the Injection taken by the formula:

$$0.5(C / V)(A_U / A_S)$$

in which *V* is the volume, in mL, of Injection taken.



## Desoxycorticosterone Acetate Pellets

» Desoxycorticosterone Acetate Pellets are sterile pellets composed of Desoxycorticosterone Acetate in compressed form, without the presence of any binder, diluent, or excipient. They contain not less than 97.0 percent and not more than 103.0 percent of  $C_{23}H_{32}O_4$ .

**Packaging and storage**—Preserve in tight containers suitable for maintaining sterile contents, holding one pellet each.

**USP Reference standards** (11)—  
USP Desoxycorticosterone Acetate RS

**Solubility in alcohol**—A solution of 25 mg of powdered Pellets in 1 mL of alcohol is clear and practically free from insoluble residue.

### Identification—

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

*Solution:* 10  $\mu$ g per mL.

*Medium:* alcohol.

Absorptivities at 240 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 155° and 161°.

**Specific rotation** (781S): between +171° and +179°.

*Test solution:* 10 mg, undried, per mL, in dioxane.

**Sterility Tests** (71): meet the requirements.

**Weight variation**—Weigh 5 Pellets singly, and calculate the average weight. The average weight is not less than 95% and not more than 105% of the labeled weight of  $C_{23}H_{32}O_4$ , and each Pellet weighs not less than 90% and not more than 110% of the labeled weight of  $C_{23}H_{32}O_4$ .

### Assay—

**Standard preparation**—Prepare as directed under Assay for Steroids (351), using USP Desoxycorticosterone Acetate RS.

**Assay preparation**—Weigh and finely powder not fewer than 10 Pellets. Weigh accurately about 100 mg of the powder, dissolve it in sufficient alcohol to make 200.0 mL, and mix. Transfer 5.0 mL of this solution to a 250-mL volumetric flask, dilute with alcohol to volume, and mix. Transfer 20.0 mL of this solution to a glass-stoppered, 50-mL conical flask.

**Procedure**—Proceed as directed for Procedure under Assay for Steroids (351). Calculate the quantity, in mg, of  $C_{23}H_{32}O_4$  in the portion of Pellets taken by the formula:

$$10C(A_U / A_S).$$

## Desoxycorticosterone Pivalate

$C_{26}H_{38}O_4$  414.58

Pregn-4-ene-3,20-dione, 21-(2,2-dimethyl-1-oxopropoxy)-, 11-Deoxycorticosterone pivalate [808-48-0].

» Desoxycorticosterone Pivalate contains not less than 97.0 percent and not more than 103.0 percent of  $C_{26}H_{38}O_4$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed, light-resistant containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—  
USP Desoxycorticosterone Pivalate RS

### Identification—

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

*Solution:* 20  $\mu$ g per mL.

*Medium:* methanol.

Absorptivities at 241 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Melting range** (741): between 200° and 206°.

**Specific rotation** (781S): between +155° and +163°.

*Test solution:* 10 mg per mL, in dioxane.

**Loss on drying** (731)—Dry it at 105° for 2 hours: it loses not more than 0.5% of its weight.

### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of methanol and water (4:1). Make adjustments if necessary (see System Suitability under Chromatography (621)).

**Internal standard solution**—Transfer about 100 mg of desoxycorticosterone acetate to a 50-mL volumetric flask, add methanol to volume, and mix.

**Standard preparation**—Transfer about 12.5 mg of USP Desoxycorticosterone Pivalate RS, accurately weighed, to a 25-mL volumetric flask, add 20 mL of methanol, and mix. Add 2.5 mL of *Internal standard solution*, dilute with methanol to volume, and mix to obtain a solution having a known concentration of about 0.5 mg of USP Desoxycorticosterone Pivalate RS per mL.

**Assay preparation**—Transfer about 50 mg of Desoxycorticosterone Pivalate, accurately weighed, to a 100-mL volumetric flask, add 80 mL of methanol, and mix. Add 10.0 mL of *Internal standard solution*, dilute with methanol to volume, and mix.

**Chromatographic system** (see Chromatography (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  25-cm column that contains packing L7. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for Procedure: the resolution,  $R$ , between the analyte and internal standard peaks is not less than 2.0; and the relative standard deviation for replicate injections is not more than 1.5%.

**Procedure**—Separately inject equal volumes (about 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. The relative retention times are about 0.5 for desoxycorticosterone acetate and 1.0 for desoxycorticosterone pivalate. Calculate the quantity, in mg, of  $C_{26}H_{38}O_4$  in the portion of Desoxycorticosterone Pivalate taken by the formula:

$$100C(R_U / R_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Desoxycorticosterone Pivalate RS in the *Standard preparation*; and  $R_U$  and  $R_S$  are the peak response ratios obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Desoxycorticosterone Pivalate Injectable Suspension

### DEFINITION

Desoxycorticosterone Pivalate Injectable Suspension is a sterile suspension of Desoxycorticosterone Pivalate in an aqueous medium. It contains NLT 90.0% and NMT 110.0% of the labeled amount of desoxycorticosterone pivalate ( $C_{26}H_{38}O_4$ ).



**IDENTIFICATION****A.**

**Sample:** Centrifuge a portion of Injectable Suspension, decant the supernatant, wash the residue by stirring with several successive portions of water, centrifuging and decanting each time, and finally dry the residue at 105°. The residue so obtained meets the following requirements.

**Analysis 1:** Melting point.

**Acceptance criteria 1:** Melts at 198°–206°.

**Analysis 2:** Dissolve 5 mg in 2 mL of sulfuric acid.

**Acceptance criteria 2:** The solution is yellowish, with a greenish fluorescence.

**Analysis 3:** Dilute the solution obtained from *Analysis 2* with 2 mL of water.

**Acceptance criteria 3:** The color changes to a dark red-blue, and on further dilution with 2 mL of water, the color is discharged.

**ASSAY****PROCEDURE**

**Mobile phase:** Methanol and water (4:1)

**Internal standard solution:** 2 mg/mL of desoxycorticosterone acetate in methanol

**Standard solution:** 0.5 mg/mL of USP Desoxycorticosterone Pivalate RS in methanol, prepared as follows. Transfer 12.5 mg of USP Desoxycorticosterone Pivalate RS to a 25-mL volumetric flask, and add 20 mL of methanol. Add 2.5 mL of the *Internal standard solution*, and dilute with methanol to volume.

**Sample solution:** Nominally 0.5 mg/mL of desoxycorticosterone pivalate in methanol, prepared as follows. Transfer a nominal equivalent of 125 mg of desoxycorticosterone pivalate from Injectable Suspension to a 250-mL volumetric flask. Add 200 mL of methanol, and sonicate to dissolve. Add 25.0 mL of *Internal standard solution*, and dilute with methanol to volume. Centrifuge a 20-mL portion at high speed for 5 min. Filter the supernatant through a 5-μm disk, discarding the first 5 mL of the filtrate.

**Chromatographic system**

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm × 25-cm; packing L7

**Flow rate:** 1.5 mL/min

**Injection volume:** 25 μL

**System suitability**

**Sample:** *Standard solution*

[NOTE—The relative retention times for desoxycorticosterone acetate and for desoxycorticosterone pivalate are about 0.5 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 2.0 between desoxycorticosterone acetate and desoxycorticosterone pivalate

**Relative standard deviation:** NMT 1.5% for the peak response ratio of desoxycorticosterone pivalate to the internal standard

**Analysis**

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of desoxycorticosterone pivalate (C<sub>26</sub>H<sub>38</sub>O<sub>4</sub>) in the portion of Injectable Suspension taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak response ratio of desoxycorticosterone pivalate to the internal standard from the *Sample solution*

$R_S$  = peak response ratio of desoxycorticosterone pivalate to the internal standard from the *Standard solution*

$C_S$  = concentration of USP Desoxycorticosterone Pivalate RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of desoxycorticosterone pivalate in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

**SPECIFIC TESTS**

• **pH** (791): 5.0–7.0

• **OTHER REQUIREMENTS:** It meets the requirements in *Injections and Implanted Drug Products* (1).

• **BACTERIAL ENDOTOXINS TEST** (85): It contains NMT 2.78 USP Endotoxin Units/mg of desoxycorticosterone pivalate.

**ADDITIONAL REQUIREMENTS**

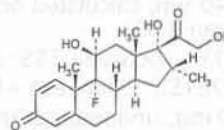
• **PACKAGING AND STORAGE:** Preserve in single-dose or multiple-dose containers, preferably of Type I glass, protected from light.

• **LABELING:** Label Suspension to indicate that it is for veterinary use only.

• **USP REFERENCE STANDARDS** (11)

USP Desoxycorticosterone Pivalate RS

USP Endotoxin RS

**Dexamethasone**

C<sub>22</sub>H<sub>29</sub>FO<sub>5</sub> 392.46

Pregna-1,4-diene-3,20-dione, 9-fluoro-11,17,21-trihydroxy-16-methyl-, (11β,16α)-.

9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione [50-02-2].

» Dexamethasone contains not less than 97.0 percent and not more than 102.0 percent of C<sub>22</sub>H<sub>29</sub>FO<sub>5</sub>, calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers.

**USP Reference standards** (11)—

USP Dexamethasone RS

**Identification—**

**A: Infrared Absorption** (197K).

**B: Ultraviolet Absorption** (197U)—

**Solution:** 10 μg per mL.

**Medium:** methanol.

Absorptivities at 239 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** (781S): between +72° and +80°.

**Test solution:** 10 mg per mL, in dioxane.

**Loss on drying** (731)—Dry it at 105° for 3 hours: it loses not more than 0.5% of its weight.

**Residue on ignition** (281): not more than 0.2% from 250 mg.

**Chromatographic purity—**

**Formate buffer**—Dissolve 1.32 g of ammonium formate in 1 L of water, adjust with formic acid to a pH of 3.6, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Formate buffer* and acetonitrile (67:33). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Test solution**—Transfer about 180 mg of Dexamethasone, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix. Transfer



about 33 mL of this solution to a 100-mL volumetric flask, dilute with *Formate buffer* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L11. The flow rate is about 1 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 5000 theoretical plates.

**Procedure**—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Dexamethasone taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity, and  $r_s$  is the sum of the responses of all peaks: not more than 1.0% of any individual impurity is found, and not more than 2.0% of total impurities is found.

#### Assay—

**Mobile phase**—Prepare a suitable degassed solution of water and acetonitrile (about 7:3) such that at an approximate flow rate of 2 mL per minute, the retention time of Dexamethasone is about 7 minutes.

**Standard preparation**—Prepare a solution of USP Dexamethasone RS in methanol having a known concentration of about 7.5 mg per mL. Dilute an accurately measured volume of this solution with the *Mobile phase* to obtain a *Standard preparation* having a known concentration of about 0.3 mg per mL.

**Assay preparation**—Using 30 mg of Dexamethasone, proceed as directed for *Standard preparation*.

**Procedure**—Introduce equal volumes (between 15 and 30 µL) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* (621)) operated at room temperature by means of a suitable microsyringe or sampling valve, adjusting the operating parameters such that the peak obtained with the *Standard preparation* is 60% full-scale. Typically, the apparatus is fitted with a 4-mm × 25-cm column containing packing L7, is equipped with an UV detector capable of monitoring absorption at 254 nm and a suitable recorder, and is operated at about 1000 psi. Five replicate injections of the *Standard preparation* show a relative standard deviation of not more than 3.0%. Determine the peak responses, at equivalent retention times, obtained with the *Assay preparation* and the *Standard preparation*, and calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in the portion of Dexamethasone taken by the formula:

$$100C(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*, and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Topical Aerosol

» Dexamethasone Topical Aerosol is Dexamethasone in a suitable lotion base mixed with suitable propellants in a pressurized container. Dexamethasone Topical Aerosol delivers not less than 90.0 percent and not more than 120.0 percent of the labeled amount of  $C_{22}H_{29}FO_5$ .

**Packaging and storage**—Preserve in pressurized containers, and avoid exposure to excessive heat.

**USP Reference standards** (11)—  
USP Dexamethasone RS

**Identification**—Evaporate 5 mL of the *Assay preparation*, obtained as directed in the *Assay*, on a steam bath just to dryness, and dissolve the residue in 1 mL of chloroform. Apply 100 µL of this solution and 10 µL of a solution of USP Dexamethasone RS in chloroform containing 500 µg per mL to a suitable thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel. Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform and diethylamine (2:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots on the plate by lightly spraying with dilute sulfuric acid (1 in 2) and heating on a hot plate or under a heat lamp until spots appear: the  $R_f$  value of the principal spot obtained from the test solution corresponds to that obtained from the *Standard solution*.

**Microbial enumeration tests** (61) and **Tests for specified microorganisms** (62)—It meets the requirements of the tests for absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

#### Change to read:

**Other requirements**—It meets the requirements for *Pressure Test*, *Minimum Fill*, and *Leakage Test* under *Topical Aerosols* (603) (CN 1-May-2017).

#### Assay—

**Standard preparation**—Prepare as directed under *Assay for Steroids* (351), using USP Dexamethasone RS.

**Assay preparation**—Shake the Topical Aerosol container gently, and invert, immersing the valve in about 75 mL of alcohol contained in a 400-mL beaker. Actuate the valve by pushing against the bottom of the beaker. Remove the container at 15-second intervals, shake gently, and allow the container to warm to room temperature. Continue spraying until the contents of the container are exhausted. Transfer the alcohol solution to a 100-mL volumetric flask, dilute with alcohol to volume, and mix. Dilute an accurately measured volume of this solution, equivalent to about 1 mg of dexamethasone, with alcohol to 100.0 mL, and mix. Transfer 20.0 mL of this solution to a glass-stoppered, 50-mL flask.

**Procedure**—Proceed as directed for *Procedure* under *Assay for Steroids* (351), except to allow to stand in the dark for 45 minutes. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in each container taken by the formula:

$$(10C / V)(A_u / A_s)$$

in which  $V$  is the volume, in mL, of assay solution taken for the second dilution.

## Dexamethasone Elixir

» Dexamethasone Elixir contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{29}FO_5$ .

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—  
USP Dexamethasone RS



**Identification**—Evaporate 9 mL of the *Assay preparation*, prepared as directed in the *Assay*, on a steam bath just to dryness, and dissolve the residue in 2 mL of a mixture of methylene chloride and methanol (1:1). Apply separately 5  $\mu$ L of this solution and 5  $\mu$ L of a solution of USP Dexamethasone RS in the mixture of methylene chloride and methanol (1:1), containing 0.5 mg per mL, to a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel mixture (see *Chromatography* (621)). Allow the spots to dry, and develop the chromatogram in a solvent system consisting of a mixture of chloroform, acetone, and glacial acetic acid (80:40:1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the developing chamber, mark the solvent front, and allow the solvent to evaporate. Locate the spots by viewing under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**Alcohol Determination, Method II** (611): between 3.8% and 5.7% of  $C_2H_5OH$ , *n*-propyl alcohol being used as the internal standard.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (2:1). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dexamethasone RS in dilute methanol (1 in 2), and dilute quantitatively, and stepwise if necessary, with dilute methanol (1 in 2) to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Elixir, freshly mixed and free from air bubbles, equivalent to about 1 mg of dexamethasone, to a 10-mL volumetric flask, dilute with water to volume, mix, and filter through a suitable membrane filter.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm  $\times$  30-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed under *Procedure*: the relative standard deviation for replicate injections is not more than 3.0%.

**Procedure**—Separately inject equal volumes (between 5  $\mu$ L and 25  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in each mL of the Elixir taken by the formula:

$$10(C/V)(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*,  $V$  is the volume, in mL, of Elixir taken, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Gel

» Dexamethasone Gel contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{29}FO_5$ .

**Packaging and storage**—Preserve in collapsible tubes. Keep tightly closed. Avoid exposure to temperatures exceeding 30°.

#### USP Reference standards (11)—

USP Dexamethasone RS

**Identification**—Evaporate 25 mL of the *Assay preparation*, prepared as directed in the *Assay*, on a steam bath just to dryness, and dissolve the residue in 0.5 mL of chloroform. The chloroform extract responds to the *Thin-Layer Chromatographic Identification Test* (201), 10  $\mu$ L of the chloroform extract and 10  $\mu$ L of a Standard solution containing about 500  $\mu$ g per mL of USP Dexamethasone RS being applied, and a mixture of chloroform and diethylamine (2:1) being used for development. Locate the spots on the plate by lightly spraying with dilute sulfuric acid (1 in 2) and heating.

**Minimum fill** (755): meets the requirements.

#### Assay—

**Mobile solvent**—Dilute 100 mL of methylene chloride with isooctane to one L.

**Chromatographic columns**—Tamp a pledget of glass wool at the constriction of a glass chromatographic tube measuring about 30-  $\times$  1.5-cm, equipped with a polytetrafluoroethylene stopcock. Fill the tube about half-full with *Mobile solvent*. Mix 8 g of chromatographic siliceous earth with 8 mL of methanol and water (1:1). Transfer successive portions of the mixture to the column, emptying and adding *Mobile solvent* after each addition to pack the column. Finally drain the column to a layer of *Mobile solvent* about 1 cm above the absorbant. Pack a second tube to provide a blank and proceed as directed for *Assay preparation*, but omit the specimen.

**Standard preparation**—Prepare a solution of USP Dexamethasone RS in alcohol to obtain a solution having a known concentration of about 10  $\mu$ g per mL.

**Assay preparation**—Accurately weigh an amount of Gel, equivalent to about 0.5 mg of Dexamethasone in a 100-mL beaker, add 1 g of chromatographic siliceous earth, and mix. Transfer the mixture to the column, wash the beaker with small portions of *Mobile solvent*, adding them to the column. Adjust the flow rate to about 2 mL per minute, discarding the eluate. Elute with four additional 25-mL portions of *Mobile solvent*, and discard. Rinse the sample beaker with two 25-mL portions of methylene chloride and transfer to the column, collecting the eluate in a suitable beaker. Elute the column with six additional 25-mL portions of methylene chloride, combining the eluates and evaporating with a gentle stream of air to dryness, dissolve the residue in alcohol, and transfer to a 50-mL volumetric flask. Wash the beaker with successive 5-mL portions of alcohol, collecting the washings in the flask, dilute with alcohol to volume, and mix. Centrifuge or filter and then pipet 10 mL of this solution, 10 mL of the *Standard preparation*, 10 mL of the column blank solution, and 10 mL of alcohol to provide a reagent blank to separate flasks. Proceed as directed under *Assay for Steroids* (351), except to allow to stand in the dark for 45 minutes. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in the portion of Gel taken by the formula:

$$(0.05C)(A_U - A_{CB} / A_S - A_{RB})$$

in which  $C$  is the concentration, in  $\mu$ g per mL, of USP Dexamethasone RS in the *Standard preparation*; and  $A_U$ ,  $A_{CB}$ ,  $A_S$ , and  $A_{RB}$  are the absorbances of the solutions from the *Assay preparation*, column blank preparation, *Standard preparation*, and reagent blank preparation, respectively.

## Dexamethasone Injection

» Dexamethasone Injection is a sterile solution of Dexamethasone in Water for Injection. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone ( $C_{22}H_{29}FO_5$ ).



**Packaging and storage**—Preserve in light-resistant single-dose or multiple-dose containers, preferably of Type I glass.

**Labeling**—Label it to indicate that it is for veterinary use only.

**USP Reference standards** (11)—

USP Dexamethasone RS

USP Endotoxin RS

**Identification**—

**A: Thin-Layer Chromatographic Identification Test** (201)—

**Test solution**—Transfer a quantity of Injection, equivalent to about 5 mg of dexamethasone, to a 50-mL separator, add 10 mL of water, and extract with two 20-mL portions of chloroform. Filter the lower layers through chloroform-saturated cotton into a 50-mL conical flask, and evaporate to dryness. Dissolve the residue in 10 mL of chloroform.

**Developing solvent system**: a mixture of methylene chloride and methanol (180:16).

**Procedure**—Visualize the spots using a 1 in 5 solution of *p*-toluenesulfonic acid in a mixture of alcohol and propylene glycol (9:1), followed by heat.

**B:** The retention time of the major peak in the chromatogram of the *Assay preparation* corresponds to that in the chromatogram of the *Standard preparation*, as obtained in the *Assay*.

**Bacterial Endotoxins Test** (85)—It contains not more than 21.0 USP Endotoxin Units per mg of dexamethasone.

**Sterility Tests** (71)—It meets the requirements when tested as directed for *Membrane Filtration under Test for Sterility of the Product to be Examined*.

**pH** (791): between 4.0 and 5.5.

**Particulate Matter in Injections** (788): meets the requirements for small-volume injections.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (70:30). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**System suitability solution**—Prepare a solution in *Mobile phase* containing in each mL about 0.3 mg of USP Dexamethasone RS, 1.35 mg of benzyl alcohol, 0.27 mg of methylparaben, and 0.03 mg of propylparaben.

**Standard preparation**—Quantitatively dissolve an accurately weighed amount of USP Dexamethasone RS in methanol to obtain a stock solution having a known concentration of about 7.5 mg per mL. Transfer 4.0 mL to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix to obtain a solution having a known concentration of about 0.3 mg of USP Dexamethasone RS per mL.

**Assay preparation**—Transfer an accurately measured volume of Injection, equivalent to about 30 mg of dexamethasone, to a 100-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-μm packing L7. The flow rate is about 2 mL per minute. Chromatograph the *System suitability solution*, and record the peak responses as directed for *Procedure*: the relative retention times are about 0.4 for benzyl alcohol, 0.5 for methylparaben, 1.0 for dexamethasone, and 1.4 for propylparaben; and the resolution, *R*<sub>s</sub> between the neighboring peaks for benzyl alcohol and methylparaben, methylparaben and dexamethasone, and dexamethasone and propylparaben is not less than 3. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 μL) of the *Standard preparation* and the *Assay preparation* into

the chromatograph, record the chromatograms, and measure the peak responses for dexamethasone. Calculate the quantity, in mg, of dexamethasone (C<sub>22</sub>H<sub>29</sub>FO<sub>5</sub>) in each mL of the Injection taken by the formula:

$$100(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*; *V* is the volume, in mL, of Injection taken; and *r<sub>U</sub>* and *r<sub>S</sub>* are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Ophthalmic Suspension

» Dexamethasone Ophthalmic Suspension is a sterile, aqueous suspension of dexamethasone containing a suitable antimicrobial preservative. It may contain suitable buffers, stabilizers, and suspending and viscosity agents. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of C<sub>22</sub>H<sub>29</sub>FO<sub>5</sub>.

**Packaging and storage**—Preserve in tight containers.

**USP Reference standards** (11)—

USP Dexamethasone RS

**Identification**—Transfer a volume of Ophthalmic Suspension, equivalent to about 2.5 mg of dexamethasone, to a test tube, add 5 mL of chloroform, and shake. Centrifuge, and apply 10 μL of the chloroform layer and 10 μL of a Standard solution of USP Dexamethasone RS in chloroform containing 500 μg per mL on a thin-layer chromatographic plate (see *Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in *Solvent A* as directed under *Single-steroid Assay* (511). Mark the solvent front, and locate the spots on the plate by spraying with a 1 in 5 solution of *p*-toluenesulfonic acid in a mixture of 9 volumes of alcohol and 1 volume of propylene glycol, and heating until spots appear. The *R<sub>f</sub>* value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

**Sterility Tests** (71): meets the requirements.

**pH** (791): between 5.0 and 6.0.

**Assay**—

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (60:40). Make adjustments if necessary (see *System Suitability under Chromatography* (621)).

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dexamethasone RS in *Mobile phase*, and dilute quantitatively, and stepwise if necessary, with *Mobile phase* to obtain a solution having a known concentration of about 0.12 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of Ophthalmic Suspension, freshly mixed and free from air bubbles, equivalent to about 3 mg of dexamethasone, to a 25-mL volumetric flask, dilute with *Mobile phase* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak response as directed for *Procedure*: the column efficiency determined from the analyte peak is not less than 1750 theoretical plates; the tailing factor for the analyte peak is not more than 3.0; and the relative standard deviation for replicate injections is not more than 3.0%.



**Procedure**—Separately inject equal volumes (about 10  $\mu$ L) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in each mL of the Ophthalmic Suspension taken by the formula:

$$25(C/V)(r_U/r_S)$$

in which *C* is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*; *V* is the volume, in mL, of Ophthalmic Suspension taken; and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Oral Solution

### DEFINITION

Dexamethasone Oral Solution contains NLT 90.0% and NMT 110.0% of the labeled amount of dexamethasone ( $C_{22}H_{29}FO_5$ ).

### IDENTIFICATION

- **A.** The retention time of the dexamethasone peak from the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

### ASSAY

#### PROCEDURE

**Mobile phase:** Methanol and water (1:1)

**Internal standard solution:** 0.1 mg/mL of USP Prednisolone RS in methanol

**System suitability stock solution:** 0.6 mg/mL of USP Methylparaben RS and 0.075 mg/mL of USP Propylparaben RS in methanol

**System suitability solution:** 0.24 mg/mL of USP Methylparaben RS, 0.03 mg/mL of USP Propylparaben RS, and 0.01 mg/mL of USP Prednisolone RS prepared as follows. To an amount of *System suitability stock solution* equivalent to 40% of the final volume, add an amount of *Internal standard solution* equivalent to 10% of the final volume. Dilute with water to volume.

**Standard stock solution:** 0.2 mg/mL of USP Dexamethasone RS in *Mobile phase*

**Standard solution:** 0.02 mg/mL of USP Dexamethasone RS and 0.01 mg/mL of USP Prednisolone RS in *Mobile phase* prepared by diluting suitable volumes of *Standard stock solution* and *Internal standard solution*

**Sample solution:** Nominally equivalent to 0.02 mg/mL of dexamethasone from a volume of Oral Solution and 0.01 mg/mL of USP Prednisolone RS in *Mobile phase* from the *Internal standard solution*

#### Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 3.9-mm  $\times$  30-cm; packing L1

**Flow rate:** 1.4 mL/min

**Injection volume:** 10  $\mu$ L

#### System suitability

[NOTE—The relative retention times for methylparaben, prednisolone, propylparaben, and dexamethasone are about 0.43, 0.71, 0.88, and 1.0, respectively.]

**Samples:** *System suitability solution* and *Standard solution*

#### Suitability requirements

**Resolution:** NLT 2.0 between methylparaben and prednisolone; NLT 2.0 between propylparaben and prednisolone, *System suitability solution*

**Tailing factor:** NMT 2.0 for each peak, *System suitability solution* and *Standard solution*

**Relative standard deviation:** NMT 2.0% for the peak height ratio of dexamethasone to prednisolone, *Standard solution*

#### Analysis

**Samples:** *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of dexamethasone ( $C_{22}H_{29}FO_5$ ) in the portion of Oral Solution taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U) \times 100$$

$R_U$  = peak height ratio of dexamethasone to prednisolone from the *Sample solution*

$R_S$  = peak height ratio of dexamethasone to prednisolone from the *Standard solution*

$C_S$  = concentration of USP Dexamethasone RS in the *Standard solution* (mg/mL)

$C_U$  = nominal concentration of dexamethasone in the *Sample solution* (mg/mL)

**Acceptance criteria:** 90.0%–110.0%

### OTHER COMPONENTS

- **ALCOHOL DETERMINATION, Method II (611)** (if present): 27.0%–33.0%

### PERFORMANCE TESTS

- **UNIFORMITY OF DOSAGE UNITS (905):** Meets the requirements for oral solution packaged in single-unit containers
- **DELIVERABLE VOLUME (698):** Meets the requirements for oral solution packaged in multiple-unit containers

### SPECIFIC TESTS

- **PH (791):** 2.7–4.0

### ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers. Store at controlled room temperature.
- **LABELING:** Label concentrated Oral Solution to state that the term “Concentrate” is to appear apart from and immediately after the official title in prominent boldface type. Label concentrated Oral Solution also to indicate that it is to be diluted to appropriate strength with a suitable diluent prior to administration unless produced for dispensing with instructions for administration by a calibrated dropper or syringe.
- **USP REFERENCE STANDARDS (11)**
  - USP Dexamethasone RS
  - USP Methylparaben RS
  - USP Prednisolone RS
  - USP Propylparaben RS

## Dexamethasone Tablets

» Dexamethasone Tablets contain not less than 90.0 percent and not more than 110.0 percent of the labeled amount of  $C_{22}H_{29}FO_5$ .

**Packaging and storage**—Preserve in well-closed containers.

#### USP Reference standards (11)—

USP Dexamethasone RS

**Identification**—Evaporate 10 mL of the methanol extract of Tablets obtained as directed under *Assay preparation* in the *Assay* on a steam bath just to dryness, and dissolve the residue in 1 mL of chloroform. Apply 10  $\mu$ L of this solution and 20  $\mu$ L of a solution of Dexamethasone RS in chloroform containing 500  $\mu$ g per mL on a thin-layer chromatographic plate (see *System Suitability under Chromatography* (621)) coated with a 0.25-mm layer of chromatographic silica gel mixture. Develop the chromatogram in *Solvent A* as directed under *Single-Steroid Assay* (511). Mark the solvent front, and



locate the spots on the plate by visualizing under short-wavelength UV light: the  $R_f$  value of the principal spot obtained from the solution under test corresponds to that obtained from the Standard solution.

#### Dissolution (711)—

**Medium:** dilute hydrochloric acid (1 in 100); 500 mL.

**Apparatus 1:** 100 rpm.

**Time:** 45 minutes.

**Standard solution**—Prepare as directed for *Standard Preparation* under *Assay for Steroids* (351), using USP Dexamethasone RS.

**Procedure**—Extract a filtered aliquot of *Dissolution Medium*, equivalent to about 200 µg of dexamethasone, with three 15-mL portions of chloroform. Evaporate the combined chloroform extracts on a steam bath just to dryness, cool, and dissolve the residue in 20 mL of alcohol. Proceed as directed for *Procedure* under *Assay for Steroids* (351), except to allow to stand in the dark for 45 minutes. Calculate the portion, in mg, of  $C_{22}H_{29}FO_5$  dissolved by the formula:

$$10(C/V)(A_U/A_S)$$

in which  $V$  is the volume, in mL, of the aliquot extracted with chloroform.

**Tolerances**—Not less than 70% ( $Q$ ) of the labeled amount of  $C_{22}H_{29}FO_5$  is dissolved in 45 minutes.

**Uniformity of dosage units** (905): meet the requirements.

**Procedure for content uniformity**—

**Standard solution**—Prepare as directed for *Standard Preparation* under *Assay for Steroids* (351), using USP Dexamethasone RS.

**Test solution**—Place 1 Tablet in a separator with 15 mL of water, and swirl to disintegrate the Tablet completely. Extract with four 10-mL portions of chloroform, filtering each portion through chloroform-washed cotton into a 50-mL volumetric flask, add chloroform to volume, and mix. Pipet a volume of this solution, equivalent to about 200 µg of dexamethasone into a glass-stoppered, 50-mL conical flask, evaporate the chloroform on a steam bath just to dryness, cool, and dissolve the residue in 20.0 mL of alcohol. Use this where *Assay Preparation* is specified in the *Procedure*.

**Procedure**—Proceed as directed for *Procedure* under *Assay for Steroids* (351), except to allow to stand in the dark for 45 minutes. Calculate the quantity, in mg, of total steroids, as  $C_{22}H_{29}FO_5$ , in the Tablet by the formula:

$$(C/V)(A_U/A_S)$$

in which  $V$  is the volume, in mL, of the aliquot taken to prepare the *Test solution*.

#### Assay—

**Mobile solvent**—Prepare a suitable aqueous solution of acetonitrile, approximately 1 in 3, such that the retention time of dexamethasone is between 3 minutes and 6 minutes.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dexamethasone RS in dilute methanol (1 in 2) to obtain a solution having a known concentration of about 0.1 mg per mL.

**Assay preparation**—Weigh and finely powder not fewer than 10 Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of dexamethasone, transfer to a 50-mL volumetric flask, and add 30 mL of dilute methanol (1 in 2). Sonicate the flask for about 2 minutes, shake by mechanical means for 30 minutes, and dilute with the same solvent to volume. Filter a portion of the mixture through a suitable filter to obtain a clear filtrate.

**Procedure**—Introduce equal volumes (between 5 µL and 25 µL) of the *Assay preparation* and the *Standard preparation* into a high-pressure liquid chromatograph (see *Chromatography* (621)) operated at room temperature, by means of a

loop injector, adjusting the specimen size and other operating parameters such that the peak obtained with the *Standard preparation* is about 0.6 full scale. Typically, the apparatus is fitted with a 4.6-mm × 30-cm column packed with packing L1 and is equipped with an UV detector capable of monitoring absorption at 254 nm and a suitable recorder. In a suitable chromatogram, the coefficient of variation for five replicate injections of a single specimen is not more than 3.0%. Measure the responses of the peaks, at identical retention times, obtained with the *Assay preparation* and the *Standard preparation*. Calculate the quantity, in mg, of  $C_{22}H_{29}FO_5$  in the portion of Tablets taken by the formula:

$$50C(r_U/r_S)$$

in which  $C$  is the concentration, in mg per mL, of USP Dexamethasone RS in the *Standard preparation*, and  $r_U$  and  $r_S$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

### Dexamethasone Acetate

$C_{24}H_{31}FO_6 \cdot H_2O$  452.51

Pregna-1,4-diene-3,20-dione, 21-(acetyloxy)-9-fluoro-11,17-dihydroxy-16-methyl-, (11β,16α)-monohydrate.

9-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 21-acetate monohydrate [55812-90-3].

Anhydrous 434.51 [1177-87-3].

» Dexamethasone Acetate contains one molecule of water of hydration or is anhydrous. It contains not less than 97.0 percent and not more than 102.0 percent of  $C_{24}H_{31}FO_6$ , calculated on the dried basis.

**Packaging and storage**—Preserve in well-closed containers. Store at 25°, excursions permitted between 15° and 30°.

**Labeling**—Label it to indicate whether it is hydrous or anhydrous.

**USP Reference standards** (11)—

USP Dexamethasone Acetate RS

#### Identification—

**A:** Infrared Absorption (197M).

**B:** Ultraviolet Absorption (197U)—

**Solution:** 15 µg per mL.

**Medium:** methanol.

Absorptivities at 239 nm, calculated on the dried basis, do not differ by more than 3.0%.

**Specific rotation** (781S): between +82° and +88°.

**Test solution:** 10 mg per mL, in dioxane.

**Loss on drying** (731)—Dry it in vacuum at 105° for 3 hours: the hydrous form loses between 3.5% and 4.5%, and the anhydrous form not more than 0.4%, of its weight.

**Residue on ignition** (281): not more than 0.1%.

#### Delete the following:

• **Heavy metals, Method II** (231): not more than 0.002%.

• (Official 1-Jan-2018)

#### Chromatographic purity—

**Format buffer**—Dissolve 1.32 g of ammonium formate in 1 L of water, adjust with formic acid to a pH of 3.6, and mix.

**Mobile phase**—Prepare a filtered and degassed mixture of *Formate buffer* and acetonitrile (3:2). Make adjustments if



necessary (see *System Suitability* under *Chromatography* (621)).

**Test solution**—Transfer about 200 mg of Dexamethasone Acetate, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with acetonitrile to volume, and mix. Transfer about 40 mL of this solution to a 100-mL volumetric flask, dilute with *Formate buffer* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 4.6-mm × 25-cm column that contains packing L11. The flow rate is about 1 mL per minute. Chromatograph the *Test solution*, and record the peak responses as directed for *Procedure*: the column efficiency is not less than 5400 theoretical plates.

**Procedure**—Inject a volume (about 10 µL) of the *Test solution* into the chromatograph, record the chromatogram, and measure the peak responses. Calculate the percentage of each impurity in the portion of Dexamethasone Acetate taken by the formula:

$$100(r_i / r_s)$$

in which  $r_i$  is the peak response for each impurity; and  $r_s$  is the sum of the responses of all the peaks: not more than 1.0% of any individual impurity is found; and not more than 2.0% of total impurities is found.

#### Assay—

**Mobile phase**—Prepare a filtered and degassed mixture of water and acetonitrile (550:450). Make adjustments if necessary (see *System Suitability* under *Chromatography* (621)).

**pH 6.0 Buffer solution**—Transfer 3 mL of 1 N sodium hydroxide, 138 mL of 0.5 N potassium chloride, and 50 mL of 0.5 M monobasic potassium phosphate to a 1-L volumetric flask, dilute with water to volume, and mix.

**Diluent**—Prepare a mixture of acetonitrile and pH 6.0 Buffer solution (1:1).

**Standard preparation**—Transfer about 25 mg of USP Dexamethasone Acetate RS, accurately weighed, to a 250-mL volumetric flask. Add 100 mL of *Diluent*, and sonicate until a clear solution is obtained. Dilute with *Diluent* to volume, and mix.

**Assay preparation**—Transfer about 25 mg of Dexamethasone Acetate, accurately weighed, to a 250-mL volumetric flask. Add 100 mL of *Diluent*, and sonicate until a clear solution is obtained. Dilute with *Diluent* to volume, and mix.

**Chromatographic system** (see *Chromatography* (621))—The liquid chromatograph is equipped with a 254-nm detector and a 3.9-mm × 30-cm column containing 10-µm packing L1. The flow rate is about 2 mL per minute. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure*: the capacity factor,  $k'$ , is not less than 2.0; the column efficiency is not less than 1500 theoretical plates; the tailing factor is not more than 2.0; and the relative standard deviation for replicate injections is not more than 2.0%.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* (before and after injections of the *Assay preparation*) and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of  $C_{24}H_{31}FO_6$  in the portion of Dexamethasone Acetate taken by the formula:

$$250C(r_u / r_s)$$

in which  $C$  is the concentration, in mg per mL, of USP Dexamethasone Acetate RS in the *Standard preparation*; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.

## Dexamethasone Acetate Injectable Suspension

» Dexamethasone Acetate Injectable Suspension is a sterile suspension of Dexamethasone Acetate in Water for Injection. It contains an amount of dexamethasone acetate monohydrate ( $C_{24}H_{31}FO_6 \cdot H_2O$ ) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of dexamethasone ( $C_{22}H_{29}FO_5$ ).

**Packaging and storage**—Preserve in single-dose or multiple-dose containers, preferably of Type I glass.

**USP Reference standards** (11)—

USP Dexamethasone Acetate RS

USP Endotoxin RS

**Identification, Infrared Absorption** (197M)—Obtain the test specimen as follows. Transfer the contents of a well-shaken container of Injectable Suspension to a fine-porosity, sintered-glass vacuum filter, filter, and wash with several 10-mL portions of water. Remove the powder from the filter and allow to air-dry. [NOTE—Do not use heat to dry the specimen. Total or partial dehydration may occur. Use a similar undried preparation of USP Dexamethasone Acetate RS.]

**Bacterial Endotoxins Test** (85)—It contains not more than 21.7 USP Endotoxin Units per mg of dexamethasone acetate.

**pH** (791): between 5.0 and 7.5.

**Other requirements**—It meets the requirements under *Injections and Implanted Drug Products* (1).

#### Assay—

**Mobile phase, pH 6.0 Buffer solution, Diluent, and Chromatographic system**—Proceed as directed in the *Assay* under *Dexamethasone Acetate*.

**Standard preparation**—Dissolve an accurately weighed quantity of USP Dexamethasone Acetate RS in *Diluent* to obtain a solution having a known concentration of about 0.09 mg per mL.

**Assay preparation**—Transfer an accurately measured volume of well-shaken Injectable Suspension, equivalent to about 40 mg of dexamethasone, to a 100-mL volumetric flask. Add 75 mL of *Diluent*, and sonicate until a clear solution is obtained. Dilute with *Diluent* to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, dilute with *Diluent* to volume, and mix.

**Procedure**—Separately inject equal volumes (about 20 µL) of the *Standard preparation* (before and after injections of the *Assay preparation*) and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity, in mg, of dexamethasone ( $C_{22}H_{29}FO_5$ ) in each mL of the Injectable Suspension taken by the formula:

$$(392.47 / 434.51)(500C / V)(r_u / r_s)$$

in which 392.47 and 434.51 are the molecular weights of dexamethasone and anhydrous dexamethasone acetate, respectively;  $C$  is the concentration, in mg per mL, of USP Dexamethasone Acetate RS in the *Standard preparation*;  $V$  is the volume, in mL, of Injectable Suspension taken; and  $r_u$  and  $r_s$  are the peak responses obtained from the *Assay preparation* and the *Standard preparation*, respectively.